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PRODUCING DEHYDRATED MEATS BY DRUM-CABINET, RETORT-CABINET, AND OTHER METHODS[1]

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The vacuum-rotary, plate-vacuum, and kettle-cabinet methods of meat dehydration were discussed in the preceding paper in this series. It also dealt briefly with the types of meat used in the investigations and the subject of yields of raw lean and dehydrated product. The present paper is concerned with five additional processes termed (1) drum-cabinet, (2) retort-cabinet, (3) air-rotary, (4) drum-air flotation, and (5) freezing-cabinet. For descriptions of the equipment used the reader is referred to the first article in the series.[1]

DRUM-CABINET PROCESS

In these experiments the double-drum dehydrator was used for precooking the meat and cabinet driers for the dehydration proper. Factors studied were (a) size of particles of the raw meat, (b) temperature of the drums as controlled by steam pressure, (c) duration of precooking period as controlled by rate of revolution of the drums, (d) temperature of the air in cabinet drier, (e) time of grinding (before or before and after precooking), (f) relative humidity of air in cabinet drier, and (g) direction of air movement in cabinet drier (across or through trays).

The general procedure in processing by the drum-cabinet method consisted in grinding the meat, placing it gradually in the trough formed by the two heated, revolving drums, collecting the precooked meat in pans as it was scraped off the drums, loading onto screen trays at a specified weight per square foot of tray surface, and drying in the cabinet machine at a certain air temperature and relative humidity.

It is obvious that in their passing between the two hot drums there is a tendency for all particles of meat, regardless of size, to be reduced to equal thickness. One might reason that this would result in consider-

[1] This is the third in a series of papers dealing with the Emergency Meat Dehydration Investigations of the United States Department of Agriculture. The first article, on equipment, and the second article, concerned also with methods for producing dehydrated meats, appeared in the September-October, 1945, issue of Food Research.

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[4] The authors wish to give credit to R. L. Hiner of the staff of the Bureau of Animal Industry for supervising slaughter of the animals and preparation of the meat for processing.

able uniformity in the time required for the precooked meat to reach a given moisture content subsequently in the cabinet drier. However, three lots of beef ground through the 0.25-inch plate and precooked on the double-drum machine required a mean of 112 minutes to reach the 10-per cent moisture level, whereas for two comparable lots ground through the 1.125-inch plate 315 minutes were necessary.

In studies of the drum-cabinet process an important problem related to the effect of different drum temperatures on the rate of subsequent drying in the cabinet. To throw light on this question, temperatures of 152.8, 155.6, 157.8, 162.2, 166.1, and 170°C. (307, 312, 316, 324, 331, and 338°F.), obtained by using steam pressures of 60, 65, 70, 80, 90, and 100 pounds, respectively, in the drums were used in various comparisons. In general an increase in steam pressure resulted in a marked decrease in drying time. For example, in one experiment steam pressures of 80 and 100 pounds were used. Beef of approximately five per cent fat content was ground through the 1.125-inch plate and precooked as indicated on the drums for about 34 seconds. This means that the drums were making one revolution in about 45 seconds. Subsequently the meat was dehydrated to nine per cent moisture content at an air temperature of 71.1°C. (160°F.) in cabinet Drier A. Periods of time required, corresponding to the steam pressures of 80 and 100 pounds, were approximately 200 and 100 minutes. In another instance, pork of about nine per cent fat content was processed under the same conditions except that the three subsamples were drum precooked at steam pressures of 60, 70, and 80 pounds. To attain a moisture content of approximately 9.3 per cent in the product, respective cabinet-drying periods of 205, 150, and 130 minutes were required.

Closely associated with temperature of the double drums during precooking is the duration of the precooking period, as determined by the rate of revolution of the drums. In one instance in the study on this factor, beef ground through the 1.125-inch plate and precooked for 15 seconds on the drums under 100 pounds' steam pressure was subsequently dried to 7.5 per cent moisture content in air at 160°F. in 160 minutes. A companion lot of meat, similarly processed except that it was in contact with the drums for 34 seconds, required only 95 minutes of subsequent drying to reach about the same moisture level. Three lots of beef, in another test, were ground through the 1.125-inch plate and precooked on the drums at 100 pounds' steam pressure for 23, 42, and 48 seconds. Subsequent drying to about 5.5 per cent moisture content was done at 160°F. in 160, 130, and 110 minutes, respectively. As still a third example, two lots of pork were processed as in the cases of the foregoing examples, but with drum precooking periods of 15 and 42 seconds. The respective drying periods necessary to attain a moisture content of approximately eight per cent were 115 and 98 minutes. It is obvious from these results that shorter drying periods in the cabinet were associated with longer precooking periods on the drums.

Several series of tests were conducted to study drying rate as affected by different drying temperatures following uniform conditions of precooking (Table 1). It is readily seen that in general the higher the temperature

TABLE 1

Drying Time for Meat Dehydrated by Drum-Cabinet Process, as Affected by Temperature of Drying Air

Type of cabinet dehydrator	Kind of meat	Lot No.	Temperature of drying air (dry bulb thermometer)	Drum conditions			Particle size (diameter of holes in grinder plate)	Approximate fat content of raw meat	Time required to reduce moisture content of meat to 10 per cent
				Time per revolution	Steam pressure	Space between drums			
			°F.	*sec.*	*lb.*	*in.*	*in.*	*pct.*	*min.*
A	Beef	575	140	65	65	.125	0.25	5.0	255
		559	160	65	65	.125	0.25	5.0	210
		571	200	65	65	.125	0.25	7.0	135
A	Beef	595	180	45	70	.10	1.125	9.0	420[1]
		597	200	45	70	.10	1.125	9.0	370[1]
A	Pork	584	140	45	70	.10	0.25	11.0	390
		586	160	45	70	.10	0.25	11.0	285
		582	200	45	70	.125	0.25	11.0	210
B	Beef	697	140	45	70	.10	0.25	13.0	165[1]
		679	160	45	70	.10	0.25	13.0	150
		700	200	45	70	.10	0.25	13.0	120
B	Pork	495	140	65	100	.125	0.25	7.5	255
		496	160	65	100	.125	0.25	7.5	240
		498	180	65	100	.125	0.25	7.5	250
		499	200	65	100	.125	0.25	7.5	155
		500	220	65	100	.125	0.25	7.5	160
B	Pork	512	140	65	100	.125	0.25	11.0	255
		513	160	65	100	.125	0.25	11.0	265
		514	180	65	100	.125	0.25	11.0	195
		515	200	65	100	.125	0.25	11.0	130
		516	220	65	100	.125	0.25	11.0	175
B	Pork	585	140	65	100	.10	0.25	10.0	330
		587	160	65	100	.10	0.25	10.0	270
		583	200	65	100	.10	0.25	10.0	180
B	Pork	618	140	45	70	.10	0.25	20.0	360[1]
		621	160	45	70	.10	0.25	22.0	165
		624	180	45	70	.10	0.25	20.0	170
		631	200	45	70	.10	0.25	20.0	195
B	Pork	633	140	45	70	.10	1.125	18.0	480
		638	180	45	70	.10	1.125	19.0	360
B	Pork	644	140	65	100	.10	1.125	13.0	600
		647	160	65	100	.10	1.125	14.0	375
		648	180	65	100	.10	1.125	14.0	360
		649	200	65	100	.10	1.125	14.0	310
B	Pork	683	140	45	70	.10	0.25	13.0	255
		681	160	45	70	.10	0.25	11.5	150
		674	180	45	70	.10	0.25	11.5	210
		672	200	45	70	.10	0.25	12.0	180

[1] Estimated.

of the circulating air in the dehydrator, the more rapid was the rate of drying. In dehydrating Lots 495 to 500 the relative humidity in the drying chamber was maintained at the high level of 45 per cent. As a result of this procedure, it will be noted, there were no important differences in drying time related to the temperatures of 60, 71.1, and 82.2°C. (140, 160, and 180°F.). When air at 93.3°C.(200°F.) was used there was a striking reduction in time requirement but no further reduction in that respect was associated with the increase to 104.4°C.(220°F.). It is likely that the air at that high temperature, despite the high relative humidity, "case hardened" or seared the meat. The same explanation appears applicable to the difference between Lots 515 and 516.

Lots 618, 621, 624, and 631 (Table 1) are of special interest on account of their high fat content. Although there was little difference in drying rate associated with the difference between 160 and 180°F. air, both of these temperatures more than doubled the rate of drying obtained with 140°F. air. The slower drying at 200°F. than at 160 or 180°F. apparently was due to greater fat encasement of the meat particles at the highest temperature. The reason for the relatively rapid drying at 160°F. in the last series (Table 1) was not determined.

In preparing dehydrated meat of the granular type, the processor is confronted with the problem as to the time of grinding in relation to the cooking. The chief consideration is the effect of this factor on rate of dehydration. One set of three drum-cabinet samples, ground only before cooking, required 150, 270, and 245 minutes to dry to 10 per cent moisture content, whereas three comparable lots ground both before and after cooking required 115, 185, and 155 minutes. By grinding the meat after as well as prior to precooking an average of 70 minutes was saved in the time required for drying to 10 per cent moisture content. This was more than 30 per cent.

Cabinet Dehydrator B was so equipped as to provide opportunity for making a study of drying time as affected by the relative humidity of the atmosphere in the dehydration compartment. Pork of 8.5 to 11.0 per cent fat content was ground through the 0.25-inch plate. Precooking was done on the double drums set 0.125-inch apart, turning at the rate of 0.92 r.p.m., and under 100 pounds of steam pressure. The various samples were dehydrated in atmospheres of 10, 20, 30, 40, 50, and 60 per cent relative humidity and in all cases at 160° F. (dry bulb) temperature. Ranging from 180 to 310 minutes, respectively, in time required to reduce the moisture content to 10 per cent, the values appeared consistently in increasing order of magnitude. The obvious conclusion was that for economical operation a low relative humidity is essential.

Several tests were conducted to throw light on the question concerning the relative advantages of air circulation across the trays and through the trays in cabinet drying. In one of them, for example, pork was ground through the 0.25-inch plate and precooked on the drums set 0.125 inch apart, turning at the rate of 0.92 r.p.m. and under 100 pounds' steam pressure. Dehydration of the two subsamples was then carried out in cabinet Drier B, using 180°F. (dry bulb) air, with circulation across the trays in one case and through the trays in the other instance. The respec-

tive periods required to dehydrate the products to approximately 10 per cent moisture content were 300 minutes and 155 minutes. In another test high-fat pork was ground through the 0.25-inch plate and precooked on the drums set 0.10 inch apart, turning at 1.33 r.p.m., and under 70 pounds' steam pressure. Two subsamples were then dehydrated in cabinet Drier B, utilizing 180°F. (dry bulb) air. Again the air circulation in one instance was across the trays and in the other through the trays. The moisture content of 10 per cent was attained in 195 and 170 minutes, respectively. It is apparent these results support the claims that circulation of air through the trays and meat promotes the more rapid drying.

RETORT-CABINET PROCESS

Pressure cooking is another possible method for the pretreatment of meat in dehydration. This method was used in conjunction with cabinet drying in the investigations and the process termed "retort cabinet."

In two experiments a study was made of the influence of fat content of the raw meat on the time required for drying (Table 2). Meat containing 11 to 12 per cent fat was compared with meat of 17 per cent fat content in Experiment A. Pork in pieces approximating two-inch cubes was cooked in shallow pans in the retort at 15 pounds' steam pressure for 60 minutes. The juice, including the fat that cooked out of the meat, was not reincorporated.

After cooking, the meat was ground through the 0.25-inch plate, spread on screen trays at the rate of 1.5 pounds per square foot, and dried in

TABLE 2

Drying Time for Meat Dehydrated by Retort-Cabinet Process as Affected by Fat Content of Raw Meat and Length of Precooking Period[1]

Experiment	Kind of meat	Lot No.	Approximate fat content of raw meat	Precooking in retort		Temperature of drying air (dry bulb thermometer)	Time required to reduce moisture content to 10 per cent
				Steam pressure	Time		
Effect of Fat Content of Raw Meat							
			pct.	*lb.*	*min.*	*°F.*	*min.*
A	Pork	686	11	15	60	180	115
		688	12	15	60	180	105
		703	17	15	60	180	215
B	Pork	630	15	15	120	160	160
		754	16	15	120	160	65
		710	24	15	120	165	255[2]
Effect of Length of Precooking Period							
C	Lamb	612B	9	19	30	180	144
		613B	9	19	45	180	115
		613A	9	19	60	180	125
C	Lamb	612A	9	19	120	180	120
		611	9	16	120	180	95
		Mean	....	....	....	....	108

[1] Particle size (diameter of holes in grinder plate) was 0.25 inch. [2] Estimated.

cabinet Dehydrator A, with the air at 180°F. The two low-fat lots dried to 10 per cent moisture content in an average of 110 minutes, whereas the high-fat sample required approximately twice as much time. The high-fat meat contained about one-third more fat on the dry basis than the low-fat meat.

In Experiment B (Table 2) both precooking and drying conditions were somewhat different from those in the other experiment. Yet the high-fat meat again required the much longer period of time to dry to 10 per cent moisture content.

In another experiment light was thrown on the length of precooking period as a factor affecting drying rate. Lamb carcasses were cut into pieces weighing approximately two pounds. These pieces were pressure cooked at 125°C.(257°F.) for different periods of time (Table 2). After cooking, the meat was boned, trimmed, and the lean portion ground through a 0.25-inch plate. The juice and fat that cooked out of the meat were not reincorporated. The ground meat was spread on screen trays at the rate of 1.5 pounds per square foot and dried in cabinet Dehydrator A at 180°F. It is apparent that drying rate tended to increase as the precooking period increased, with the meat autoclaved for two hours drying to 10 per cent moisture content in an average of approximately 1.75 hours. Actually, however, there was not a great deal of variation in drying time related to the 45, 60, and 120-minute cooking periods. This suggests that, of the three, 45 minutes would probably be the most advantageous.

The generally rapid rate of drying obtained in these experiments was regarded as due to (a) elimination of some of the fat and moisture during the pressure cooking, and (b) grinding after cooking.

PRETREATMENT METHODS COMPARED

Several general methods of pretreatment have been discussed in conjunction with cabinet dehydration. At this point it is appropriate to consider the relative merits of these methods as they affect the rate of drying in the cabinet. In the following comparisons the conditions employed in the application of each general method may be regarded as representative of that method.

Comparable lots of pork were pretreated as follows:

(1) Ground through 0.25-inch plate and cooked in kettle at 100°C. (212°F.) for 45 minutes.
(2) Cooked in kettle in two-inch pieces at 212°F. for 45 minutes and ground through 0.25-inch plate.
(3) Ground through 0.25-inch plate and cooked on double drums, separated 0.10 inch, at 70 pounds' steam pressure, for 34 seconds.
(4) Cooked in retort in two-inch pieces at 118.3°C.(245°F.) for 60 minutes and ground through 0.25-inch plate.

After the treatments indicated, all lots of meat were air-dried at 180°F. with relative humidity that did not exceed 31 per cent. The circulation of air was across the trays.

The retort-cooked meat dried to 10 per cent moisture content the most rapidly, 110 minutes being required. The rapid drying was undoubtedly due to the combined effects of the loss of some fat and juice in the cooking

and the grinding after cooking. To reach the 10 per cent moisture level only about 10 minutes more time was required by the meat that was kettle cooked and then ground. This result indicated that the fat and juice loss in the case of the retort-cooked meat was not an important factor.

When the meat was ground first and then kettle-cooked, instead of the reverse, nearly twice as much time was required for dehydration. In the case of grinding followed by cooking on the double drum a period of 300 minutes was necessary for drying to the 10-per cent level. The tendency for this method of pretreatment to sear or "case harden" the meat particles was regarded as the cause of the slow rate.

AIR-ROTARY PROCESS

The steam-tube machine, described in an earlier paper, was the first of two air-rotary dehydrators studied. The first group of tests involved precooking in the retort. Factors of primary consideration were cylinder pitch and speed, steam pressure in, or temperature of, the steam tubes, rate of feeding, extent of air recirculation, and air direction.

Based on preliminary work, the decision was made to use 65 pounds of steam pressure in the tubes, the minimum pitch possible or one inch over the six-foot length of the cylinder, and have the latter rotate at its slowest speed, 2.4 r.p.m.

The rate of feeding was established through the use of several lots of pork of about the same composition. After precooking under 15 pounds' steam pressure in all cases, the meat was ground through the 0.125-inch plate. With the damper half open and air movement counter to that of the meat, the first lot was fed into the dehydrator at the rate of 15 pounds per hour, the second 7.5 pounds, and the third 5.0 pounds. As discharged from the machine after 120 minutes of drying the respective lots of meat contained 22, 11, and 6 per cent moisture. These results indicated that the proper rate of feeding of meat so pretreated and dehydrated was about 7.0 pounds per hour, to give a product of 10 per cent moisture content.

With respect to recirculation of air, further work showed that more rapid drying occurred when the damper was almost completely closed than when one-half or three-fourths open. The closed damper caused the air to be largely recirculated and to be replaced in the system slowly, with less heat loss.

Continuous operation is always of definite interest. It appeared possible through utilizing double-drum cooking prior to air-rotary dehydration. Pork of 15 per cent fat content was ground through the 0.125-inch plate and cooked on the drums for about 22 seconds, with the latter under 70 pounds' steam pressure and set one-sixteenth of an inch apart. The meat was then reground through the 0.125-inch plate and fed into the steam-tube machine at the rate of five pounds per hour. Pressure in the tubes was 65 pounds. Air direction was countercurrent, that is, opposite the movement of the meat, and the damper was half open. The cylinder of the drier was set at the minimum pitch. Under these conditions the meat dried to about nine per cent moisture content with one passage through the machine.

The steam-jacketed kettle was used for precooking a number of lots of pork which were subsequently dried in air-rotary Dehydrator A. It was

concluded that the optimum rate of feeding was about five pounds of precooked meat per hour with the steam-tube pressure at 65 to 70 pounds, cylinder pitch at the minimum, air direction countercurrent, and the damper practically closed.

Other tests showed the fat content of the raw meat to be an important factor. Pork containing 24 per cent fat was slow in drying, whereas another lot, of 29 per cent fat content, failed to discharge properly from the machine, owing to adhering to the inside of the cylinder. Raw meat trimmed to a fat content of not more than 18 per cent dried at a suitable rate when processed by the kettle air-rotary method.

Comparison of parallel and countercurrent air flow through the steam-tube-equipped cylinder showed that the latter promoted the more rapid drying.

The other machine of air-rotary type, also described in the first paper of this series, differed from Drier A in the manner of heat transference to the meat. Whereas Drier A was equipped with steam tubes in the revolving cylinder, in Drier B use was made of heated air blown through the tumbling meat. As the cylinder revolved, the air entered the drying chamber through louvers in the inner concentric shell. Owing to limited time, the work done with this machine was mainly concerned with rate of feeding.

On the basis of preliminary tests, it was decided to operate air-rotary Dehydrator B at one r.p.m., with the dampers so adjusted as to take in little fresh air and exhaust only extremely wet air. Air heated to about 240°F. was blown into the revolving cylinder at the rate of 400 c.f.m. The natural pitch of the cylinder was 2.5 inches over the six-foot length. Thermocouples were so placed that the temperature of the meat could be determined at the wet, middle, and dry ends.

Meats dehydrated in Drier B were first cooked in a steam-jacketed kettle. The adopted procedure was to cook the meat at 212°F. for about 10 minutes, drain off the liquid, separate the fat from the juice, add the latter back to the meat in the kettle, and resume the cooking so that it covered a total period of 30 to 40 minutes. The meat was then ground.

The first lot of pork (No. 799) was fed into the machine at the rate of 0.5 pound per minute (Table 3). The air blew the meat particles so forcefully that they were carried through without drying sufficiently. The air intake opening was then reduced to the point where little of the meat was blown from the cylinder and the partially dried sample, No. 799, was fed in again. The moisture content was 3.3 per cent as the product came from the machine.

Nevertheless it was believed advisable to use as high air velocity as possible. In the next run (No. 801, Table 3) an initial load of 55 pounds of cooked meat was used. It formed a sort of cushion which reduced the air velocity below that which would blow any undried meat out of the machine. A feeding rate of 0.5 pound per minute was then maintained which, after 20 minutes, was increased to one pound. About 25 pounds of the initial load placed into the drier overflowed the dam at the end and was discharged in a few minutes only partially dried. Half-hourly moisture determinations showed that a feeding rate of one pound of meat

TABLE 3

Drying Time for Pork, Precooked in Kettle and Dehydrated in Air-Rotary Dehydrator B as Affected by Various Factors[1]

Lot No.	Time of precooking	Temperature during dehydration		Initial load in dehydrator	Rate of feeding per minute	Moisture content at 30-minute intervals during dehydration					
		Range	Average			30	60	90	120	150	180
	min.	*°F.*	*°F.*	*lb.*	*lb.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
799	30	.....	.....	0	0.50	....	....	....	....	....	....
801	40	100-212	153	55	0.5-1.0	28	14.0	4	2.5	3.0	....
802	40	110-200	146	25	1.5	34	10.5	10	11.0	7.5	....
805	40	105-212	146	0	0.62	48	31.0	13	2.5	3.0	3
810	40	108-240	168	0	0.75	15	14.0	....	....	....	....
817	40	100-190	128	0	1.50	32	20.0	24	16.0	....	....

[1] Temperature of meat in precooking was 100°C.(212°F.). Particle size (diameter of holes in grinder plate) was 0.25 inch.

per minute was too slow, since in 1.5 hours the meat discharged was of four per cent moisture content. This suggested the use of a smaller initial load and a higher rate of feeding. Therefore, of Lot 802, 25 pounds were fed into the machine initially, followed by a feeding rate of 1.5 pounds of meat per minute. This procedure gave very satisfactory results from the processing point of view. In one hour the meat being discharged contained 10.5 per cent moisture. It appeared probable that a feeding rate of 1.3 pounds per minute would be optimum under this set of conditions. In air-rotary dehydration the objective is to establish such a feeding rate, under any given set of conditions, that the discharged meat is of 10 per cent moisture content at most, yet not so low that it is economically undesirable or that the meat becomes overheated or scorched.

In further study of the alternative method, which involved reduction of the air velocity to such an extent that no wet meat would be blown out of the cylinder, Lot 805 was processed with the use of a feeding rate of 0.62 pound of cooked meat per minute. After two hours of dehydration the moisture content of the meat being discharged was 2.5 to 3.0 per cent.

With all conditions comparable to those used for Lot 805 except rate of feeding, Lot 810 was fed at the rate of 0.75 pound and Lot 817 at 1.50 pounds per minute. The results indicated that the former rate was close to the optimum, whereas the latter was definitely excessive.

From these experiments it seems that either a feeding rate of 1.5 pounds per minute with initial load of 25 pounds, or a rate of 0.75 pound per minute starting with an empty machine could be used. The former, of course, would be the more practicable.

The results indicated further that in using a machine of commercial size the operator would be able to establish a set of conditions which would produce a product dehydrated to a moisture content of 10 per cent or below. Once these factors were established a product of uniform composition should be turned out from day to day.

Tremendous advantages of air-rotary driers are the continuity of the processing and the relatively small crew required. Apparent disadvantages are that particular care must be taken to keep the machine clean and that the air and relatively high temperature used and contact with hot metal surfaces in the dehydration tend to cause fat oxidation and instability.

DRUM-AIR FLOTATION PROCESS

Designed by a member of the Department staff,[5] the air-flotation dehydrator was built at the Beltsville (Md.) Research Center. It is described in the first article of this series.

The usual procedure was to precook ground meat on the double-drum drier and to follow with dehydration in the air-flotation machine. The process, therefore, was termed "drum-air flotation." The factors studied in relation to drying rate were (a) fat content of raw material, (b) weight of load, (c) temperature of drying air, and (d) size of particle.

Relatively high fat content of the raw material did not greatly retard the rate of drying, as shown by two comparable sets of tests, in one of

[5] W. A. Noel.

which the mean fat content was 9.1 per cent and in the other 12.7 per cent. All lots of meat were ground through the 0.25-inch plate and precooked on the drums at about 70 pounds' steam pressure. The respective mean periods of time on the drums were 34 and 30 seconds and the mean temperature of the air in the flotation chamber was about 78.3°C.(173°F.) in both cases. With final moisture percentages of 10 and 8.8 for the low and high fat groups, respectively, the mean periods of time required for drying were 109 and 135 minutes. Two other comparable sets of tests gave a similar result. The small decrease in drying rate associated with higher fat content of raw material was probably influenced by the brushing off of some free fat from the swirling meat particles against the inside wall of the flotation chamber. This appeared to keep fat encasement of the particles to a moderate level.

In the air-flotation dehydrator 30-pound lots of pork dried, on the average, to 9.1 per cent moisture content in 138 minutes. Fifty-pound lots, which were processed under the same conditions, dried to approximately the same moisture content in the same mean period of time. It would appear from these results that the extent to which the load might be increased, without affecting the rate of drying, would depend on the volume and, possibly, velocity of air and the supply of heat.

The temperature of the air in the drying chamber had an important effect on the rate of dehydration; for example, six comparable beef samples were dehydrated at the temperatures of 48.9, 60, 71.1, 76.7, 82.2, and 93.3 to 96.1°C.(120, 140, 160, 170, 180, and 200 to 205°F.). The corresponding drying periods were 270, 255, 180, 150, 125, and 77 minutes, with the moisture content of the finished products varying from 7.2 to 9.8 per cent. It is noteworthy that at the intermediate air temperature of 160°F. the meat was dehydrated to a moisture content of 9.4 per cent in 180 minutes.

That size of particle is a factor which affects drying rate in the drum-air flotation process was shown by two comparable lots of beef ground through the 0.375 and 0.875-inch plates. With precooking done under identical conditions, the particles of smaller size dried to 8.2 per cent moisture content in 90 minutes in 148°F. air, whereas those of larger size were reduced only to 11.6 per cent moisture in 115 minutes at the higher air temperature of 165°F.

FREEZING CABINET

One of the objectives in the dehydration of any food is to produce a product that will closely approach the fresh food in appearance and eating qualities. To attain that objective is probably more than should be expected when the food is precooked, heated to a relatively high temperature in the drying, and cooked again in preparation for eating. It was logical, therefore, that an interest should develop on the part of the investigators in the dehydration of raw meat or in processing at temperatures below the coagulation point of meat protein.

A number of lots of coarsely ground pork were frozen overnight or longer at —17.8 or —23.3°C.(0 or —10°F.), reground with one exception through the 0.25-inch plate, and dried in cabinet Dehydrator B at air

temperatures of 26.7 to 60°C. (80 to 140°F.). The effect of air temperatures on the rate of drying was studied with four of these lots. All were frozen at 0°F., reground through the 0.25-inch plate, and in each instance all six trays of cabinet Dehydrator B were loaded at the rate of approximately 1.5 pounds of meat per square foot. Circulation of the air was through the trays. At 80°F. the time required to dehydrate to 2.4 per cent moisture content was 780 minutes. Only 300 minutes were required to dry to 3.0 per cent moisture at 140°F. The other two temperatures considered being 37.8 and 48.9°C.(100 and 120°F.), the optimum of the four appeared to be 120°F. The drying rate in that case was about the same as at 140°F. and there was clearly less possibility of protein coagulation.

Circulation of air through the trays was much more efficient than across the trays, as shown by the results from a comparable pair of lots. With conditions of freezing, grinding, and tray loading similar to those outlined above, an air temperature of 120°F. was used. The meat subjected to through circulation dried to 3.8 per cent moisture content in 257 minutes. The other lot, subjected to cross circulation, was reduced only to 8.0 per cent in 390 minutes.

Apparent assets of the freezing-cabinet methods were (a) rapid drying at relatively low temperatures which prevented, or at least minimized, protein denaturation, (b) extremely good fat retention by the product, (c) rapid rehydration of the product, and (d) similarity of the freshly dehydrated meat to fresh meat in appearance and flavor. Liabilities are the possible presence of bacterial toxins and enzymes in the raw dehydrated product. The problems relating to the first of these factors is yet to be solved, although incomplete results indicate that it is not especially serious or difficult. Enzymes not inactivated can have a marked effect on keeping quality. It is probable that only through a moisture content perhaps as low as two per cent could enzymatic action be sufficiently retarded to give good keeping quality in a vacuum pack at normal temperatures. Further studies are required on this and related points.

SUMMARY AND CONCLUSIONS

Meat-dehydration methods discussed in this paper are the drum-cabinet, retort-cabinet, air-rotary, drum-air flotation, and freezing-cabinet.

Generally, in the drum-cabinet process, smallness of meat particle, high drum temperature, relatively long precooking period, grinding after as well as before cooking, and high air temperature with low relative humidity and through circulation of air in the cabinet contributed to the more rapid drying. Representative conditions for this method include grinding meat through the 1.125-inch plate, cooking on the double drums, separated 0.10 inch, at 170°C.(338°F.) (100 pounds' steam pressure) for 48 seconds, regrinding through the 0.25-inch plate, spreading the meat on screen trays at the rate of 1.5 pounds per square foot, and drying in a cabinet with through circulation of air heated to 82.2°C.(180°F.) and at a relative humidity not exceeding 31 per cent.

Precooking in the retort-cabinet process removed some fat and juice from the meat. Nevertheless, high fat content of raw material reduced the rate of dehydration in the cabinet. Duration of precooking had little effect

on the time required for drying. Representative processing conditions included heating the meat in pieces under pressure only until cooked throughout, grinding through the 0.25-inch plate, loading on screen trays at the rate of 1.5 pounds per square foot, and dehydrating in circulating air heated to 180°F. Even 71.1°C.(160°F.) air effected rapid drying. Air circulation through the trays and control of relative humidity at a low level would have further accelerated the dehydration.

Of four pretreatment methods compared, retort cooking followed by grinding resulted in the most rapid dehydration. Kettle cooking followed by grinding was a close second. Drum cooking preceded by grinding led to the slowest drying.

For Machine A, in air-rotary processing, the optimum rate of feeding was about five pounds of precooked meat per hour, with the cylinder set at a pitch of one inch over the six-foot length and rotating 2.4 r.p.m., and with 65 to 70 pounds' steam pressure in the tubes. Countercurrent air circulation was better than parallel, and recirculation of most of the air was advantageous. Meat of high fat content tended to adhere to the hot steam tubes. An initial charge of 25 pounds of precooked meat followed by 1.5 pounds per minute, proved the most practical method of feeding Machine B. In contrast with Machine A, which mainly utilized contact with steam tubes for drying, Machine B employed rapidly moving, heated air that passed through the tumbling meat particles.

In the drum-air flotation process, higher fat content of raw material did not greatly retard dehydration. Neither did increased size of load, so long as sufficient air and heat were available to "float" the meat and keep it at the desired temperature. Large particles dried more slowly than small particles. Higher temperatures caused very rapid drying but even at 160 and 180°F. it was at a relatively high rate. This process involved maximum exposure of the meat to air and, therefore, to oxygen.

Freezing followed by grinding and cabinet drying at air temperatures ranging from 26.7 to 60°C.(80 to 140°F.) were involved in the freezing-cabinet process. The temperature of 48.9°C. (120°F.) appeared to be optimum, and air circulation through the trays was more efficient than cross circulation. In its freshly dehydrated form, appearance and flavor of the product closely approached fresh meat. Further studies are needed on enzyme inactivation, directed toward better keeping quality especially at temperatures above normal.

PRELIMINARY INVESTIGATION ON MEASUREMENT OF COLOR IN CANNED FOODS[1]

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Color is the most evident and frequently the most important factor in the description of canned foods. Although much progress has been made on the identification of the plant pigments and their role in plant physiology, comparatively little has been done toward measuring the relative amount of these pigments in samples of prepared foods.

The pigments involved in the color expression of vegetables are the chlorophylls, carotinoids, and anthocyanins. For a discussion of the identity, separation, and determination of the above pigments in their various isomers, reference is made to the volume by Strain (1942) and to Zscheile (1941).

Factors affecting the color of canned vegetables are operative first on the raw product. Hereditary varietal differences are important. Currence (1930) established the hereditary factors controlling the color of stringless beans, and Lindstrom (1926) has reported similar work on tomatoes. Magruder and Wester (1940) reported on the inheritance of green color in the cotyledon of the lima bean. Wide variations in color among beet varieties are illustrated by Magruder, Boswell, *et al.* (1940a), while Miller, Cochran, and Garrison (1934) and Werner (1941) found variations in color among carrot varieties.

With some vegetables, the stage of maturity of the raw product is closely related to color; thus, as the tomato fruit matures the original green turns to a whitish green as the chlorophyll disintegrates, then to a light yellow as carotin begins to develop, and finally through orange to a red color as the lycopin increases in quantity, Hansom (1921). The best color, therefore, was found by MacGillivray (1928) in tomatoes that have completely ripened. Most lima bean varieties in commercial use at present turn white as they mature. So close has the relation between maturity and color of lima beans been considered, that color has been generally used as an indication of maturity, according to Raymaley, Ball, and White (1941). Since the new green-seeded strains are coming into general use, however, this method of determining maturity will no longer be satisfactory. Emsweller, Burrell, and Borthwick (1936) and Werner (1941) found that the color of carrots improved with maturity, and apparently

[1] Contribution from the Labeling Committee, National Canners' Association, and the Horticulture Department, Maryland Agricultural Experiment Station (Contribution No. 1969).

a similar relationship exists in the development of the yellow pigment in yellow sweet corn. On the other hand, there appears to be little effect of maturity on color of beets and stringless beans, Culpepper (1936).

Seasonal effects are influential, particularly when maturity is not effective; thus Magruder, Boswell, *et al.* (1940a) illustrate wide variations in the color of beets. That the seasonal effect on beets is primarily a temperature effect has been shown by the work of Murray (1929) and others. The influence of temperature on the color of tomatoes has been studied by MacGillivray (1935), Duggar (1913), Hansom (1921), and Went, LeRosen, and Zechmeister (1942).

Canning procedures also have their effect on the final color of the canned product. The effect of canning procedure on tomato color is discussed by Hansom (1921), MacGillivray (1931, 1933), Matlack and Sando (1933), and Mozingo (1926). The effect of processing on stringless beans and peas, and probably on most other green vegetables, is to change the chlorophyll to pheophytin and thus cause some browning, according to Blair and Ayres (1943). The effect of canning procedures on the resultant color of canned beets is discussed by Blair (1936).

METHODS OF COLOR MEASUREMENT

In developing a method for measuring color in prepared foods, it was not considered necessary to determine quantitatively the amount of actual pigment present. The relative intensity of the total color or of some critical color fraction can be used as a numerical notation of the color for practical color evaluations. The ultimate criterion of the value of any such method is, of course, its relation to organoleptically determined color ratings evaluated statistically.

The practicability of the method for use by the average canner was also considered. The few previous attempts to measure the color of canned foods were not entirely satisfactory for two main reasons: first, the instruments previously available were too complex or too cumbersome for general use; and second, these instruments that were not too complex were not sufficiently accurate, especially for measuring borderline cases. More recently, however, we have been fortunate in having the use of more refined instruments, which greatly increase accuracy and at the same time are much simpler to operate.

The instruments used fall into two general categories which may be classified as (1) psychophysical, that is, instruments which give a numerical value of the color impression as seen by the eye of the observer, and (2) physical, that is actual physical measures in terms of wave length and per cent of color transmittance at a given wave length. In these studies one instrument of each type was used.

Disc Colorimeter: A Keuffel and Esser disc colorimeter was used. This particular instrument has the advantage of a built-in light source which can be adjusted accurately and a revolving lens which picks up the color impression from the color discs which remain stationary and may therefore be moved while the machine is in operation. For a detailed description of the Munsell Color System, reference is made to Cleland (1921); Munsell

Color System (1940, 1943); Symposium on color (1941); Nickerson (1945); and the bibliographies contained in some of these papers. For a description of the disc colorimeter used, reference is made to Nickerson (1931).

The following procedure was used to establish a color test when the disc colorimeter was employed: (1) The three or four color cards of a known Munsell notation were selected, which when combined matched the color of the product. (2) Several samples selected for their wide variation in color were tested in order to check on the range available with the particular color cards. (3) A large number of samples was tested with the machine, and a duplicate set was rated organoleptically by competent observers in order to establish the simplest satisfactory method of expressing the results, and also to suggest objective limits for color designations.

Spectrophotometer: This method involves the extraction of the color from the product by the use of one or more solvents and the measurement of that color solution in the spectrophotometer in terms of per cent transmittance of light at a specific wave length. A Coleman Universal spectrophotometer was used for these studies.

In establishing a spectrophotometric test, the procedure used was as follows: (1) A suitable solvent for extracting the desired pigment was selected. (2) The pigment solution was examined for turbidity, since turbidity interferes with transmittance measurement. (3) The transmittance curve was plotted against wave length for several samples varying widely in color in order to find the wave length showing the widest range for the particular pigment, and in order to establish the optimum dilution of the color extract. (4) A large number of samples were tested at the selected wave length and compared with duplicates rated organoleptically by competent observers in order to arrive at suggested limits for color designations.

The results may be expressed simply as per cent transmittance, with the reference as 100 per cent. Thus the greater the intensity of the pigment, the lower the per cent transmittance.

COLOR MEASUREMENT IN CANNED TOMATOES

The color of canned tomatoes is usually based on an examination of the drained tomatoes. The liquid in canned tomatoes also has considerable tomato color and this may require evaluation. The standard of quality for canned tomatoes under the Food, Drug, and Cosmetic Act includes a minimum color requirement based on the poorer colored half of the drained tomatoes. The test is made after pulping of these drained tomatoes.

Disc Colorimeter: In order to make possible the measurement of semiliquid products with the Keuffel and Esser colorimeter, it was necessary to devise a cell in which to place the sample. For this purpose a rectangular jar of clear glass was devised, five inches wide, five inches high, and one inch from front to back. The top was left open. This cell holds the drained tomatoes from a No. 2 can. When the drained sample is packed solidly in the color cell, first one vertical surface and then the other may be examined and the result averaged. Four color discs of

glossy surface, specially developed for canned tomatoes, and specified by the Munsell notation of 5R2.6/13 (red), 2.5YR5/12 (yellow), N1 (black), and N4 (gray), were found to give satisfactory reading over a wide range of color. The results presented (Table 1) agree with the conclusion of MacGillivray (1928) that value, that is, the amount of gray or black, is not important. When only the factor of hue was considered and the relative amount of red and yellow was expressed as R/Y index, a close relationship was found to obtain between this index and color as determined organoleptically (Table 1).

TABLE 1

Relation of Color Ratings to R/Y Index in Canned Tomatoes

Color rating	Number of samples averaged	Per cent of discs				R/Y index[5]
		Red[1]	Yellow-red[2]	Black[3]	Gray[4]	
Excellent	3	73	10	13	4	7.3
Very good	2	69	11	14	6	6.3
Good	6	66	13	14	7	5.1
Average	4	64	15	13	8	4.3
Fair	9	63	20	12	5	3.2
Poor	1	55	26	14	5	2.1

[1]Munsell notation 5R2.6/13. [2]Munsell notation 2.5YR/12. [3]Munsell notation N1. [4]Munsell notation N4. [5]Red (5R2.6/13) over yellow (2.5YR/12).

Not only is the general color of the tomatoes in the can important but also the variability of the color of the tomatoes within the can. Thus, in order to obtain some measure of the variability, samples similarly treated were read for color in their entirety. A lens on the colorimeter was then changed so that a reading could be made on an area only one inch in diameter. Color determinations were then made on such small areas in the center of the cell and on four corners of the cell. Five additional readings were made on the other surface of the cell. The average of the red-over-yellow index of the two readings of the entire sample was considered as the entire sample color, and the mean and standard deviation of the color determination on the smaller areas from the color of the entire sample were calculated. Since it was pointed out[2] that values obtained by dividing the percentage of the yellow into percentage of the red would tend to overemphasize differences in color among the better samples and underemphasize color differences among the poorer samples, additional values for these same samples were calculated by dividing the percentage of red by the percentage of red plus twice the percentage of yellow. The R/R+2Y index was, therefore, calculated for the entire sample as well as for the small diameter reading and the mean and standard deviations from the entire sample were also calculated.

Data for the above (Table 2) show that the standard deviation for the R/R+2Y index appears to be the best of the four mathematical expressions of variability. Even that value, however, appears to overemphasize color differences among the good samples.

[2] Miss Dorothy Nickerson, Color Specialist, U. S. Department of Agriculture.

In another attempt at measuring the variability within a can of tomatoes the poorest colored third of each of the drained samples was first separated and a color reading obtained for it separately as well as for the remainder of the sample (Table 3). Here also the R/R+2Y index appears to be better than the R/Y index, and again differences between the better samples are overemphasized.

TABLE 2

Variability of Canned Tomato Color as Determined by Deviations of Spot Readings With Disc Colorimeter From Readings of Total Sample

Color description		Number of samples averaged	R/Y index[1]			R/R+2Y index (x10)[2]		
Quality	Uniformity		Index	Mean dev. ±	Std. dev. ±	Index	Mean dev. ±	Std. dev. ±
Very good	Uniform	3	6.1	1.6	1.9	7.5	.54	0.64
Good	Uniform	3	5.3	1.5	1.9	7.2	.70	0.96
Fair	Variable	2	3.3	1.0	1.6	6.2	.70	0.96
Poor	Uniform	1	2.1	0.5	0.6	5.0	.60	0.77
Poor	Variable	2	2.4	1.0	1.1	5.5	.94	1.14

[1]Percentage of the red disc divided by percentage of the yellow. [2]Percentage of the red disc divided by percentage of the red plus twice the percentage of the yellow disc, multiplied by 10.

TABLE 3

Variability of Canned Tomato Color as Determined by Disc Colorimeter Readings on Poorest Third Compared With Better Two-Thirds of Sample

Color description		Number of samples averaged	R/Y index		R/R+2Y index (x 10)	
Quality	Uniformity		Better ⅔	Poorest ⅓	Better ⅔	Poorest ⅓
Very good	Uniform	2	7.3	5.1	7.8	7.1
Good	Uniform	1	6.1	3.6	7.5	6.6
Fair	Uniform	1	3.5	2.4	6.4	5.4
Fair	Variable	1	3.7	1.6	6.5	4.4
Poor	Variable	1	2.4	1.6	5.5	4.4

Spectrophotometer: Five different methods of extracting tomato pigment were tried. The method chosen for its simplicity as well as accuracy was as follows: The drained tomatoes were blended in a Waring blendor; a five-gram sample of the blended material was transferred to another blendor cup with a minimum amount of water and about 70 milliliters of benzol and blended thoroughly for about five minutes. The contents of the blendor cup were then transferred to 100-ml. graduate cylinder. The colorless solids settle to the bottom very rapidly. After standing for several minutes the solution was made up to 100 ml. with additional benzol and a two-ml. aliquot was transferred to a 15-ml. centrifuge tube and diluted with eight ml. of benzol. The aliquot was then centrifuged and measured in the spectrophotometer at 485 mμ.

Per cent transmittance curves for the benzol extracts are shown (Fig. 1) for a well-colored can of tomatoes and a poorly colored sample. It is obvious that the wave length at which there is the greatest range is about 485 mμ.

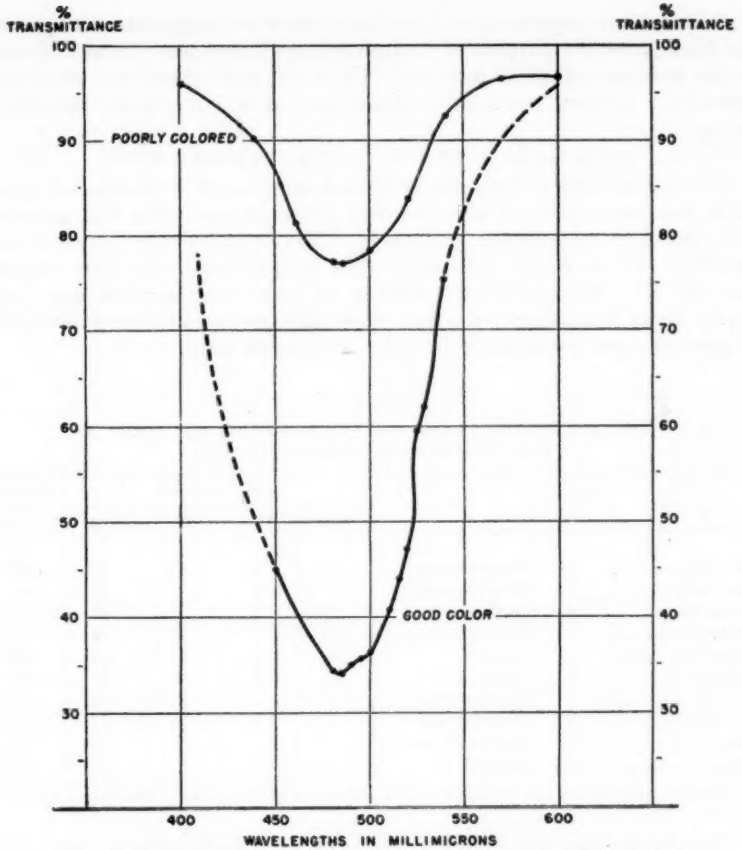

FIG. 1. Transmittance curves for benzol extracts of canned tomatoes.

TABLE 4

Relation of Color Ratings to Transmittance of Benzol Extracts in Canned Tomatoes

Color rating	Number of samples averaged	Transmittance (485 mμ)
		pct.
Excellent	3	32.8
Very good	6	36.0
Good	4	39.9
Average	3	45.3
Fair	5	48.3
Poor	3	57.1

Twenty-four samples of canned tomatoes were prepared at a commercial factory for the purpose of obtaining large differences in color. Results of the analyses of these tomatoes (Table 4) show that there is a good correlation between per cent transmittance at 485 mμ. and organoleptic ratings.

COLOR MEASUREMENT IN CANNED STRINGLESS BEANS

Disc Colorimeter: The color of canned samples of six varieties of green beans and one variety of wax beans of different maturities was measured with the disc colorimeter (Table 5). The samples were drained and placed in the usual cell. The small (one inch or less) cuts were dropped into the cell, with occasional shaking in order not to allow any large empty areas. The larger cuts and especially the whole beans needed to be pressed down occasionally in order to leave no gaps.

TABLE 5

Color Evaluations of Green and Wax Beans Obtained by Use of Disc Colorimeter and Spectrophotometer

Variety	Sieve size	Color	Per cent of discs				Transmittance (680 mμ)
			Black[1]	Gray[2]	Green[3]	Yellow[4]	
							pct.
Blue Lake	3	Bright green	36	6	12	46	81.0
Blue Lake	5	Bright green	31	6	21	42	81.5
Bountiful	6	Dark green	22	2	31	45	82.0
Tendergreen	5	Dark green	24	5	27	44	83.3
Giant Stringless	5	Green	24	8	27	41	84.3
Refugee	3	Light green	15	5	30	50	86.1
U. S. No. 5	3	Faded green	5	4	25	66	88.5
U. S. No. 5	5	Faded green	3	4	32	61	91.3
Wax	2-3	Yellow (sl. green)	....	....	....	....	96.0
Wax	6	Yellow	....	....	....	....	98.3

[1]Munsell notation N1. [2]Munsell notation N4. [3]Munsell notation GY5/6. [4]Munsell notation Y5/6.

The color discs N1 (black), N4 (gray), GY5/6 (green), and Y5/6 (yellow) were used to measure all green beans except the more mature U. S. No. 5, which are faded to such an extent that the above discs are too dark, but they may be measured readily by using neutral discs of a light value (e.g., N7 and N4). The wax beans were readily measured with the following discs: N1 (black), N4 (gray), GY7/9 (green), and Y7/9 (yellow).

The older varieties, Giant Stringless, Tendergreen, and Bountiful, are quite similar in their color analysis. The brighter color of the Blue Lake variety is apparently due to the larger percentage of yellow in proportion to green, while the faded appearance of the Refugee group of beans seems to arise from the small percentage of black. The wax beans contain approximately the same low percentage of black but very much more yellow than the Refugee group of beans.

Spectrophotometer: Canned samples of six varieties of green beans and one of wax beans of varying maturities were blended with an equal weight of water in a Waring blendor. Duplicate 20-gram aliquots were removed and made up to 150 ml. with 80 per cent alcohol. One of the sets

was brought to boiling temperature, all the samples were allowed to stand overnight and were then filtered according to the regular Alcohol Insoluble Solids procedure, made up to 300 ml., and aliquots measured on the spectrophotometer.

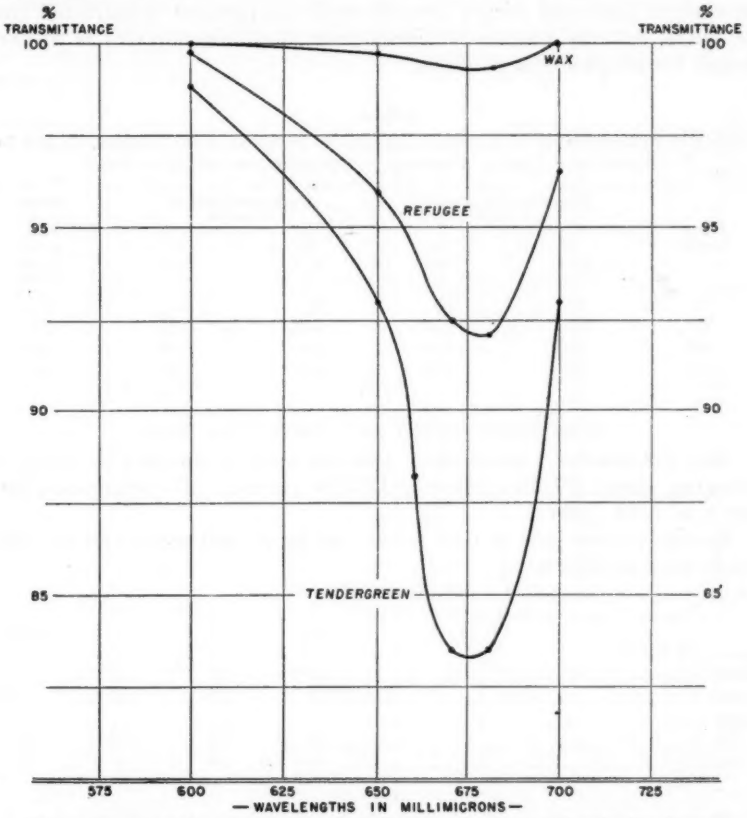

FIG. 2. Transmittance curves for alcoholic extracts of canned stringless beans.

The curve of the per cent transmittance of a deep green (Bountiful), a light green (U. S. No. 5), and a yellow (wax bean) sample at the various wave lengths of the visual spectrum (Fig. 2) show that they are practically identical in character to the curves obtained for lima beans. It is interesting to note that these curves are also almost identical to those of the alcoholic fresh-leaf tissue extracts reported by Webb and Ferguson (1941).

The filtered alcohol extracts of all the samples were measured photometrically at 400, 450, and 680 mμ. wave lengths immediately after filtering and again 24 hours later. An analysis of the results (Table 6) indicates that even though the range from the dark to the bright green sample at 680 is narrowest, the results at that wave length are so much

more uniform that the best method to follow would be to boil the sample in 80 per cent alcohol, filter, make up the filtrate to 300 ml. with additional 80 per cent alcohol, and measure at 680 mμ. wave length. Readings obtained in accordance with this procedure are presented (Table 5). It is not claimed that with such a procedure all the pigment is extracted from the solids, but the fraction removed from duplicate samples is uniform enough for all practical purposes.

TABLE 6

Errors in Transmittance of Alcoholic Extracts at Different Wave Lengths, Caused by Heating and Time of Standing of Canned Green and Wax Beans

Wave length	Heated to boil, allowed to stand		Not heated, allowed to stand		Range from dark green to pale yellow
	18 hours ±	42 hours ±	18 hours ±	42 hours ±	
mμ.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
680	0.05	0.35	1.02	1.21	17
450	1.03	1.46	1.41	1.81	29
400	0.75	0.64	3.22	3.13	44

COLOR MEASUREMENT IN CANNED LIMA BEANS

Disc Colorimeter: Satisfactory readings may be obtained by using the following discs: Y7/10 (yellow), GY7/10 (green), N7 (white, i.e., light gray), and N4 (gray).

Results on two cans of lima beans, one green and white and the other green, were as follows:

	Per cent			
	Yellow	Green	Gray	White
Green and White				
Side A	34	15	46	5
Side B	33	16	45½	5½
Green				
Side A	37	19	42	2
Side B	35	16	47	2

The lima beans were drained and dropped into the cell (as were the short cut snap beans). Addition of liquor caused a reduction in the per cent green, and especially white, and an increase in yellow and gray. A reading of the blended material can probably be obtained without difficulty, but since the object is to measure the color of the beans as they are, a measure of the color of the entire bean should be more informative. Moreover, the surface color of the lima beans may not be exactly the same as the internal color.

Spectrophotometer: Direct photometric readings of aliquots of Alcohol Insoluble Solids filtrates failed completely because of the turbidity of the filtrate which increased upon standing, and varied considerably between duplicates. Petroleum ether, in proportion of one part or more to two parts of the AIS filtrate, cleared the entire solution after thorough but gentle shaking. Vigorous shaking caused the emulsion ring, which gradu-

ally disappears between the layers of ether and alcohol, to persist for many hours. The two layers were separated. The spectrophotometric curves of per cent transmittance compared with distilled water are given (Fig. 3). For the small green limas the ether-alcohol ratio was 1:2. For the large green and the white limas the ratio was 1:1. From these curves it is evident that the best difference in transmittance is at 680 mμ. for the ether extract, while for the alcohol extract there is no comparable dip in the curve.

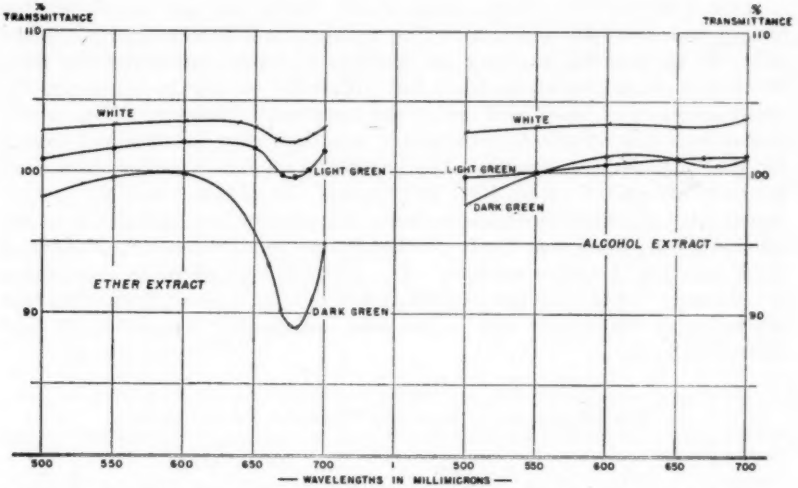

FIG. 3. Transmittance curves for ether and alcohol extracts of canned lima beans.

Data on the color and maturity of 25 samples of canned lima beans (Table 7) show that varietal differences in all tests are striking. From the disc-colorimeter data it appears that green Fordhooks are darker (less white) and more yellow than green Hendersons. The dry lima variety

TABLE 7

Color Evaluations of Canned Lima Beans Obtained by Use of Disc Colorimeter and Spectrophotometer

Number of samples averaged	Variety	Size	Color	Per cent of discs				Transmittance[5] (680 mμ)
				White[1]	Gray[2]	Green[3]	Yellow[4]	
								pct.
9	Fordhook	Medium large	Green	2	44	21	33	92.1
2	Henderson	Tiny	Green	6	45	36	13	82.6
2	Henderson	Small	Green, light	9	44	30	17	89.6
2	Henderson	Mixture	Green, light	10	42	25	23	89.3
1	Henderson	Medium	Green, pale	16	31	21	32	94.8
4	Henderson	Mixture	A third white	12	42	19	27	93.8
3	Henderson	Medium	All white	23	30	16	31	100.6
2	Dry soaks	Large	Yellow white	13	34	15	38	100.2

[1]Munsell notation N7. [2]Munsell notation N4. [3]Munsell notation GY7/10. [4]Munsell notation Y7/10. [5]Compared with distilled water at 100 per cent transmittance.

has a decidedly yellowish cast. As the Henderson lima beans mature, they become lighter and more yellow in color. A comparison of the disc colorimeter with the spectrophotometer indicates that at a wave length of 680 it is the green, rather than the yellow, pigment that absorbs the color; hence, the all-green Fordhooks appear little better by the photometric test than the green and white Hendersons.

COLOR MEASUREMENT IN CANNED BEETS

Disc Colorimeter: When using a cell similar to that developed for tomato-color work the beets must first be diced and then dropped into the cell with no packing, mashing, or addition of liquid; otherwise the mass becomes so dark that there is no red sufficiently strong to bring out the entire amounts of black and red at the same time. However, if the above precautions are observed, satisfactory readings may be obtained except for the very darkest samples. The specifications for the color discs used for beets are 4R4/6 (red), RP3/10 (purple), N1 (black), and N3 (gray). Apparently the chief variation between the color of beet samples is in the color attribute known as "value," that is, the relative amount of dark or black entering the color complex.[3] The next color attribute in importance is "chroma," or the intensity of the red color. As a result it appears that the index of black over red is the most satisfactory expression of beet color (Table 8).

TABLE 8

Relation of Color Ratings to B/R Index in Canned Beets

Number of samples averaged	Style	Color rating	Per cent of discs				B/R index[5]
			Red[1]	Purple[2]	Gray[3]	Black[4]	
2	Whole	Dark	10	2	3	85	8.5
4	Whole	Medium	13	3	2	82	6.3
2	Whole	Light	17	5	4	74	4.4
2	Whole	Very light	24	7	6	63	2.6
3	Diced	Dark	9	4	4	83	9.2
2	Diced or sliced	Medium	13	3	2	82	6.3
2	Diced or sliced	Light	16	4	4	76	4.7
1	Diced	Very light	24	7	5	64	2.7

[1]Munsell notation 4R4/6. [2]Munsell notation RP3/10. [3]Munsell notation N3. [4]Munsell notation N1. [5]Black (N1) divided by Red (4R4/6).

It is also already apparent that the size of the units of diced beets measured affect the color. The smaller the units, the darker the resultant color.

Spectrophotometer: Four cans of beets were examined. A 100-gram sample from each can was blended with an equal weight of water. Two aliquots of 20 grams of the blended material were transferred into 400-ml. beakers and one of the aliquots was made up to 300 ml. One third was centrifuged, filtered, and measured in the spectrophotometer; another third, after standing overnight, was placed in the photometer directly

[3] It is interesting to note that whereas in the case of tomato color the attribute of "hue" is most significant, with beets it is least important.

and the remainder filtered before placing in the spectrophotometer. Another 100-gram sample of beets from each can was treated similarly, except that it was blended with a one-per cent aqueous solution of thiocarbamide instead of water.

The spectrophotometric curves of per cent transmittance for Sample 1 (light), and Sample 2 (dark) are given (Fig. 4). The character of the curves is similar to those obtained by Pucher, Curtis, and Vickery (1938) for the raw product, the low point of transmission being at approximately 530 mμ.

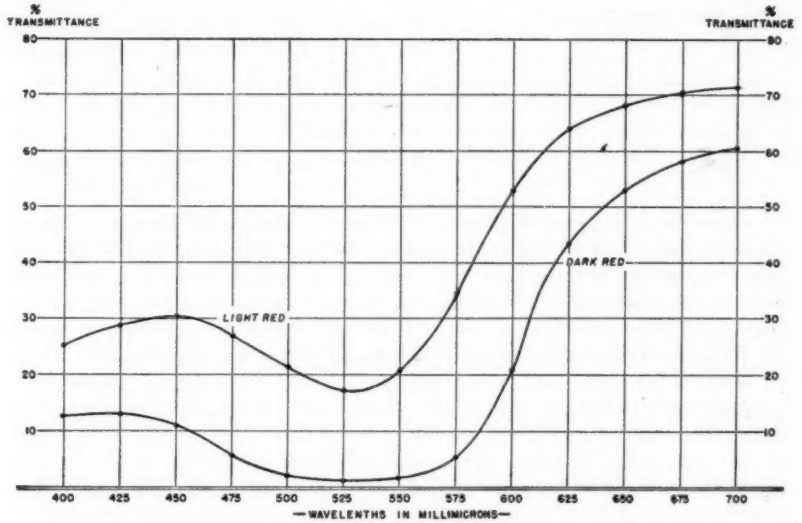

FIG. 4. Transmittance curves of water extracts of canned beets.

Readings were obtained at the wave lengths of 450, 525, and 600 mμ. There was only a slight change in values when the solution was allowed to stand for 24 hours, and practically no change at all at 525 mμ. Filtering is necessary in order to remove some suspended particles, but centrifuging may be substituted for by allowing the solution to stand for several hours. The wave length of 525 mμ. gives the most consistent results.

In order to determine the amount of dilution required to obtain a maximum range on the spectrophotometer two samples of canned beets, one of very good, bright color and the other of a faded, light red color, were blended in the usual manner. Aliquots of the two samples were then diluted to 10, 50, and 100 times the original weight with water. The results (Table 9) indicate that the greatest range between a good and poor sample may be obtained at the 100-to-1 dilution at the wave length of 525, which was previously found to be most consistent.

Data presented (Table 10) indicate that there is no large range of pH for canned beets. The samples having the lower pH values might show some tendencies toward a red rather than purple color. The effect of pH, however, appears to be unimportant in relation to the pigment concentra-

tion. The ratio of beets to liquor and the moisture content of the beets themselves, also seem to have little effect on the color.

TABLE 9

Transmittance at Different Wave Lengths for Various Dilutions of Water Extracts of Canned Beets

Sample and dilution	Wave length		
	450 mμ	525 mμ	600 mμ
	pct.	*pct.*	*pct.*
Good, bright color			
1:10	0.9	0.9	5.1
1:50	17.9	5.0	48.2
1:100	41.0	19.1	67.8
Poor, light color			
1:10	3.7	1.5	18.2
1:50	47.0	29.2	68.7
1:100	68.9	55.3	82.8

Since betanin is water soluble, the color of the liquor should be closely related to the color of the beets proper. In fact, the data (Table 10) indicate that values obtained on the color of the liquor show a wider range than those obtained on the beets. In view of probable differences in proportion of liquor to drained beets in the can, it may be necessary to adjust the liquor value for differences in volume of liquor in proportion to volume of beets in the can. Otherwise, all that would be required would be to transfer an aliquot of the liquor, dilute it 100 times with water, and measure the diluted solution in the spectrophotometer at the wave length of 525 mμ., using distilled water as a reference at 100 per cent transmittance. Results on 24 samples of canned beets analyzed in this way yielded a correlation of 0.94, thus indicating that the transmittance of liquor at 525 mμ. is in close agreement with organoleptic ratings for beet color.

TABLE 10

Relation of Color Ratings of Canned Beets to Transmittance, pH, Total Solids, and Ratio of Liquor to Beets

Color rating	Transmittance (at 525 mμ, 1:100 dil.)		pH		Weight		Total solids	
	Liquor	Beets	Liquor	Beets	Liquor	Beets	Liquor	Beets
	pct.	*pct.*			*gm.*	*gm.*		
Very dark	27.0	26.2	5.05	5.46	160	425	10.20	13.88
Dark	42.5	38.1	5.16	5.51	195	400	9.64	12.04
Medium	53.3	45.1	4.70	5.10	175	389	6.25	8.10
Medium	52.0	43.2	5.21	5.64	205	377	7.32	9.62
Light	64.0	48.9	5.10	5.52	190	408	8.25	10.74
Very light	74.1	45.7	4.91	5.39	215	355	7.06	9.12

DISCUSSION

Comparison of Disc-Colorimeter and Spectrophotometer Methods

Each method has its advantages and limitations. In the preparation of the sample the great advantage lies with the disc colorimeter in that the

sample is measured practically as it is removed from the container. Some extraction procedure is required for the photometric sample unless the pigment is water soluble.

Disc-colorimeter data show the color attributes and permit an analysis of the differences in color; however, the results are sometimes difficult to explain and compare.

The spectrophotometric method, on the other hand, is least influenced by the human element, does not require special training, can be obtained by color-blind individuals, and is less cumbersome. It is much easier to keep the cuvettes of the photometer clean than to keep thumb prints and dust particles from the color cards.

Beet color is a good example of a pigment that may be easily measured on the spectrophotometer since the pigment is water soluble and the liquor may be measured directly. Tomato color, on the other hand, might more easily be measured on the disc colorimeter because of the complexity of pigments involved.

Recently, Kelley, Gibson, and Nickerson (1943) have shown the relationship between the Munsell color cards and spectrophotometric measurements and developed relatively rapid methods for calculating spectrophotometric methods in terms of Munsell notations and vice versa. Such calculations, however, can be made only if the spectrophotometric measurements are made of the reflected color of opaque materials, whereas the measurements made above were of transparent solutions. Measurement of reflectance however does not provide opportunity for obtaining a representative sample as does measurement of transmittance where the entire sample may be first thoroughly blended.

Before using a particular spectrophotometer, it is extremely important to know the specifications of the instrument and have it adjusted properly. An instrument which gives wide bands of light will not give results comparable to those obtained with narrower bands of a more nearly monochromatic instrument. When similar pigment extracts were measured on two spectrophotometers, one a Coleman monochromator and the other a Coleman Universal, the results varied considerably. It so happened that at the wave length chosen for measuring tomato color the two instruments gave similar per cent transmittance readings, although there were considerable differences in the transmittance curves (compare Figs. 5a and 5b). For lima beans, however, the two instruments give entirely different transmittance readings, so that no comparison can be made between results obtained on one instrument and those obtained on the other (Fig. 6).

It is also possible that a series of stable solutions may be prepared for each product for the use of canners or institutions which may not have a suitable spectrophotometer. Each sample could be compared against such solutions and described accordingly.

It should be emphasized that the data presented are only of an exploratory, preliminary nature. Much more extensive sampling under variable conditions needs to be done before the suggested methods can be recommended as objective color tests for the different canned products. Further

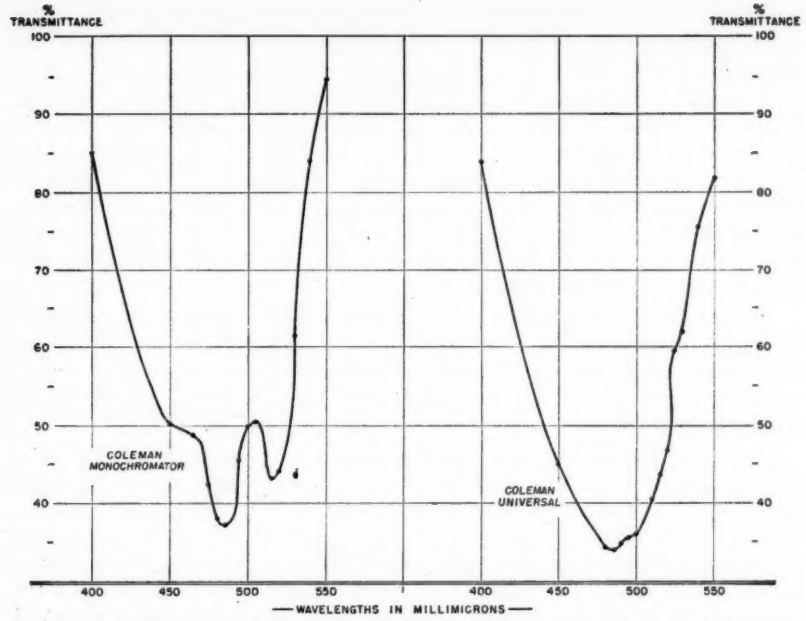

FIG. 5. Comparison of transmittance curves obtained on benzol extracts of tomatoes by using different spectrophotometers.

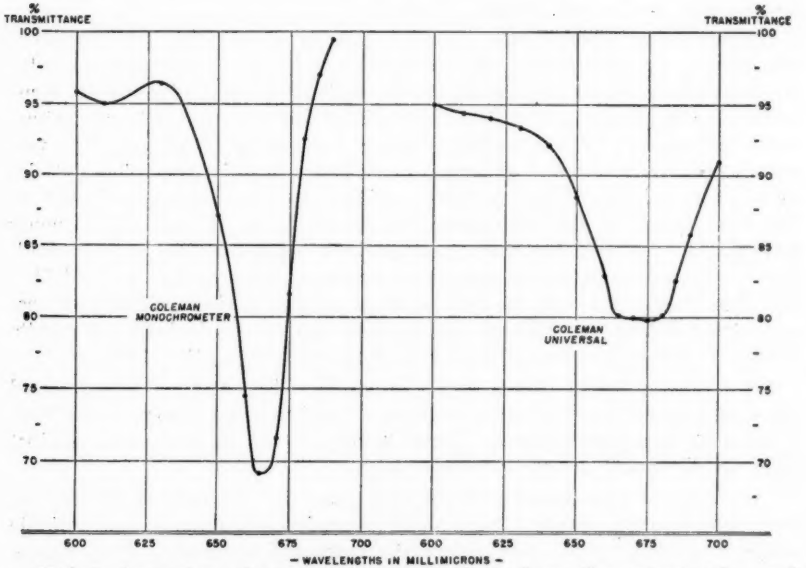

FIG. 6. Comparison of transmittance curves obtained on ether extracts of canned lima beans by using different spectrophotometers.

work is now proceeding, and more detailed reports are planned for each product separately.

SUMMARY OF TENTATIVELY ADOPTED PROCEDURES

Disc-Colorimeter Method

Tomatoes: Press drained tomatoes into a rectangular cell constructed of clear glass, five inches wide, five inches high, and one inch deep, with the top left open. Eliminate all air bubbles, compare with the glossy discs specified by the Munsell notation: 5R2.6/13 (red), 2.5YR/12 (yellow), N1 (black) and N4 (gray). Divide the per cent red by the per cent yellow and report as R/Y index.

For a measure of variability within the can, separate the drained tomatoes by hand into the better two-thirds and poorest one-third by color and measure the color of each separately using a smaller cell.

Green and Wax Beans: Drop short cuts into the cell directly; large cuts or whole pods may need to be pressed down to avoid empty areas on the cell surface. Compare the color with discs of the following Munsell notations: Y5/6 (yellow), GY5/6 (green), N1 (black), and N4 (gray). For wax beans, use the following discs: Y7/9 (yellow), GY7/9 (green), N1 (black), and N4 (gray).

Lima Beans: Fill the color cell as for short cuts of snap beans, compare with discs of the following Munsell notations: Y7/10 (yellow), GY7/10 (green), N7 (light gray), and N4 (gray).

Beets: If not diced, dice as closely as possible into ⅜-inch cubes. Drop into cell with no application of pressure and compare with discs of the following Munsell notations: RP3/10 (purple), 4R4/6 (red), N1 (black), and N3 (gray).

Carrots: Prepare as for beet samples, compare with following discs: 2YR6.3μ15 (orange), Y8/12 (yellow), N1 (black), and N5 (gray).

Spectrophotometric Method

Tomatoes: Thoroughly mix drained tomatoes by blending in a Waring blendor. To a five-gram sample add 75 ml. of benzol, blend thoroughly, make up to 100 ml. with additional benzol. Dilute by five with benzol, centrifuge, measure on the spectrophotometer at wave length of 485 mμ. with pure benzol at 100 per cent transmittance.

For a measure of variability within the can, separate out by hand one-third of the material which has the poorest color and analyze for color as above, the one-third poorest part and the two-thirds better part separately.

Green and Wax Beans: Blend the drained beans in a Waring blendor with an equal weight of water. Transfer 20 grams of the blended material to a 400-ml. beaker with 13 ml. of water and 80 per cent alcohol as required, add 150 ml. of 95 per cent alcohol, bring to boil, filter, make up filtrate to 300 ml. with additional 80 per cent alcohol and measure on the spectrophotometer at the wave length of 680 mμ.

Lima Beans: Proceed as for snap beans but make up alcohol filtrates to 250 ml., transfer 50 ml. of the alcohol filtrates to a separatory funnel, add 25 ml. of petroleum ether, shake, discard the alcohol layer, and measure the ether layer in the spectrophotometer at the wave length of 680 mμ.

Beets: For the liquor dilute 100 times with water and measure in the spectrophotometer at 525 mμ. wave length.

For drained beets, blend with an equal weight of water, dilute a 10-gram sample with 490 ml. of water, shake well, centrifuge, filter, measure filtrate in the spectrophotometer at 525 mμ. wave length.

Carrots: Proceed as for lima beans but measure on spectrophotometer at 400 mμ. wave length.

Corn: Proceed as for carrots.

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COMPRESSIBILITY OF DEHYDRATED MEAT[1]

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Early in 1942 it became apparent that there was vital need to conserve overseas shipping space and weight. This led to a consideration of processing and shipping dehydrated meat to our allies and armed forces. It was obvious that full advantage should be taken of any opportunity for compressing the product into a smaller volume after it had been dried. Moreover, compression was viewed as a means of removing air, and thereby retarding the oxidation processes which are in part responsible for the development of rancidity.

OBJECTIVES

The principal objective of this phase of the meat-dehydration study was to determine the compressibility of dehydrated meat as influenced by the original density of the product and its temperature when pressure was applied. The size, shape, and fat content of the meat particles were viewed as major factors contributing to density that required evaluation. The results of compression were to be measured in terms of changes in volume, density, texture, fat content, and rehydration capacity.

EQUIPMENT AND GENERAL PROCEDURE

In these studies a hydraulic-type laboratory press was used with a test cylinder, approximately 5.7 centimeters (2.25 inches) in diameter (inside) and 7.6 centimeters (3 inches) in depth, for holding meat samples. The area of the cross section was calculated at 25.52 square centimeters. In filling the cylinder with a selected sample, no packing was involved, the material being poured in lightly. The weight of the meat, cylinder, and base was taken and that of the sample determined by subtraction. The prescribed pressure was then applied and the sample allowed to remain under pressure for two minutes.

To determine the density of the uncompressed material its height in the test cylinder, in centimeters, was multiplied by the area of the cross section of the cylinder (25.52 square centimeters) and the resulting value for volume divided into the weight of the sample, in grams. On removal from the cylinder, after compression, the height of the sample was again determined, its weight obtained, and the density calculated by this same procedure. Differences in weight between the uncompressed and compressed sample were due to loss of fat; differences in height represented reductions in volume or in space requirements.

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[1] This paper deals with the Emergency Meat Dehydration Investigations of the U. S. Department of Agriculture. Another paper in the series appears in this issue of *Food Research*; still others have been published in an earlier issue of the journal.

[2] Transferred to Bureau of Standards, United States Department of Commerce.

Pork was used because of the suitability of available dehydrated samples and the greater interest which existed in that kind of dehydrated meat than in any other in the United States.

RESULTS

In the first comparison, a study was made of "low-fat" (27 per cent ether extract) and "high-fat" (36 per cent ether extract) pork samples, both of 0.25 inch (6.35 mm.) particle size [3] and dehydrated by the vacuum-rotary method (Table 1). Both were subjected to increasing pressures, at 75-pound intervals, up to 300 pounds per square inch (1 pound equals 0.45 kilogram and 1 square inch equals 6.45 square centimeters). In addition the "low-fat" sample was compressed at 450, 600, and 750 pounds. The work was done at room temperature of approximately 25.6°C.(78°F.).

The densities of the two samples, uncompressed, were 0.60 and 0.85. The "high-fat" sample maintained the greater density at all pressures and attained a maximum density at a much lower pressure than the "low-fat" sample. Up to 600 pounds of pressure at least, there was no fat loss from the product containing 27 per cent fat. On the other hand, the "high-fat" dehydrated meat had an appreciable loss of fat under 75 pounds of pressure and the loss increased to about 4.5 per cent when the pressure was 300 pounds. Associated with the lower original density of the "low-fat" product was a greater reduction in space requirement under a given pressure.

Another comparison, shown by Samples 564 and 494 (Table 1), was concerned with size of particle, the former having been ground through a 0.25-inch plate and the latter through a 1.125-inch (28.6 mm.) plate. Both lots were vacuum-rotary processed and contained from 36 to 38 per cent of fat. The respective densities of the dehydrated, uncompressed products were 0.85 and 0.60.

At all pressures employed the greater density was obtained in the product of the smaller particle size. This meat attained its maximum density (1.13) at about 225 pounds' pressure per square inch. The maximum density (1.04) of the other sample was attained at the same pressure. Fat losses of Sample 564 were considerably more than those of the large particle size. It is of interest that Sample 564 had a greater density (1.07) resulting from 75 pounds of pressure than had No. 494 (density of 1.04) from 225 pounds. Moreover, at these stages the fat loss of the former sample was the less.

The meat in particles of large size was reduced the more in volume when subjected to a given pressure; however, this greater indicated reduction in space requirement was associated with lower original density and lower maximum density.

Of the three types of dehydrated pork processed by the vacuum-rotary method—low fat-small particle, high fat-small particle, and high fat-large particle—the greatest density at a given pressure was attained in that of high-fat content and small-particle size. In fact, 75 pounds of pressure

[3] The holes in the plate of the grinder through which the meat was passed before dehydration were 0.25 inch in diameter.

TABLE 1

Compressibility of Dehydrated Pork[1] as Affected by Fat Content and Particle Size

Sample No. and description	Pressure per square inch on sample	Height of sample in test cylinder	Weight after compression	Fat pressed from sample		Volume	Density	Decrease in volume
	lb.	*cm.*	*gm.*	*gm.*	*pct.*	*cu. cm.*	*wt./vol.*	*pct.*
No. 588B;	0	6.0	92.0	0	....	153.0	0.60	0
fat, 27 per cent;	75	4.3	92.0	0	....	109.7	0.84	28.4
particle size,	150	3.9	92.0	0	....	99.5	0.92	35.0
0.25 inch	225	3.7	92.0	0	....	94.4	0.97	38.4
	300	3.5	92.0	0	....	89.3	1.03	41.6
	450	3.3	92.0	0	....	84.2	1.09	45.0
	600	3.2	92.0	0	....	81.7	1.13	46.6
	750	3.1	90.3	1.7	1.9	79.1	1.14	48.3
No. 564;	0	6.0	130.0	0	....	153.0	0.85	0
fat, 36 per cent;	75	4.7	128.5	1.5	1.2	120.0	1.07	21.6
particle size,	150	4.5	124.3	5.7	4.4	115.0	1.08	25.0
0.25 inch	225	4.3	124.5	5.5	4.2	110.0	1.13	28.4
	300	4.3	124.2	5.8	4.5	110.0	1.13	28.4
Number 494;	0	6.0	90.0	0	....	153.0	0.60	0
fat, 38 per cent;	75	3.7	90.0	0	....	94.4	0.95	38.3
particle size,	150	3.5	89.2	0.8	0.9	89.3	1.00	41.7
1.125 inches	225	3.3	88.0	2.0	2.2	84.2	1.04	45.0
	300	3.3	87.7	2.3	2.6	84.2	1.04	45.0

[1]Meat dehydrated by the vacuum-rotary process and compressed at approximately 25.6°C.(78°F.).

resulted in a somewhat more dense product in that instance than did 300 and 225 pounds in the cases of the low fat-small particle and high fat-large particle types, respectively. However, the high fat-small particle product lost about one per cent, owing to expressed fat, at the pressure of 75 pounds. This would be unimportant under commercial conditions if the compressing were done directly in the container.

The compressibility of four typical samples of pork dehydrated by the drum-cabinet method (Table 2) was studied as follows: (1) low-fat, small particles, (2) low-fat, large particles, (3) high-fat, small particles, and (4) high-fat, large particles. All tests were made at room temperature of approximately 78°F.

Ranging from 0.43 to 0.45, the original density of these samples was much lower than that of the vacuum-rotary samples previously discussed. This was attributed to the drum precooking which produces flaky and more or less crusty material that does not, of its own accord, pack very solidly in a container. Moreover, to some extent small particles of meat become matted together on the drums so that the distinction between them and larger particles tends to be lost.

Further, there was little variation in density among the four samples at any pressure within the range of from 75 to 600 pounds (Table 2), although up to 300 pounds the low fat-small particle sample did tend to run below the other three. The two high-fat samples appeared to attain maximum density with 450 pounds of pressure. Both low-fat samples responded to greater pressure and showed a density of 1.05 at 600 pounds.

There was noticeably less loss of fat from the samples of small-particle size; for example, No. 674 lost no weight under 450 pounds of pressure, whereas No. 692, of large particle size, lost 3.7 per cent. In the comparisons of these four samples, fat content and particle size appeared about equal in their effects on fat loss from compression.

The decrease in volume for a given pressure did not vary greatly among the four samples. However, No. 638 (Table 2) did tend to exceed the others and this was associated with serious fat losses. Up to 150 pounds of pressure, where there was no fat loss from any sample, the low fat-small particle sample (No. 674) had the least reduction in volume.

Notes on the appearance and firmness of the press cakes (Table 2) make it clear that small size of particle and high-fat content contributed to the holding of shape by the cake, which would be important under commercial conditions if the product were placed in the container after compressing. Observation indicated no great changes in the texture of the product owing to the use of pressures up to 600 pounds. However, the increase in rehydration values was believed due in part to the crushing of particles but perhaps, to a major extent, to the expressing of fat. Much the greatest increase in capacity to take up moisture occurred in the case of the high fat-large particle sample which had lost the most fat by compression.

When the same drum-cabinet samples were compressed at 3.3°C. (38°F.), the resulting densities and decreases in volume did not differ greatly from those observed at 25.6°C.(78°F.). Probably the most significant result was the small fat loss. Only from the high fat-large particle

TABLE 2

Compressibility of Dehydrated Pork[1] as Affected by Fat Content and Particle Size

Sample No. and description	Pressure per square inch on sample	Height of sample in test cylinder	Weight after compression	Fat pressed from sample		Volume	Density	Decrease in volume	Rehydration[2]	Appearance and firmness of press cake
	lb.	*cm.*	*gm.*	*gm.*	*pct.*	*cu. cm.*	*wt./vol.*	*pct.*	*gm.*	
No. 674;	0	6.0	66.3	0	....	153.0	0.43	0	30.4	.....
fat, 28 per cent;	75	4.4	66.3	0	....	112.3	0.59	27	31.8	Did not hold shape
particle size,	150	3.7	66.3	0	....	94.4	0.70	39	36.5	Partly held shape
0.25 inch	225	3.0	66.3	0	....	76.6	0.87	50	34.0	Held shape but loosely packed
	300	2.9	66.3	0	....	74.0	0.90	52	35.0	Held shape when lifted
	450	2.5	66.3	0	....	63.8	1.04	58	32.8	Medium—firm cake
	600	2.4	64.4	1.9	2.9	61.2	1.05	60	43.0	Firm cake, but particles still distinct
No. 692;	0	6.0	68.2	0	....	153.0	0.45	0	19.6	.....
fat, 26 per cent;	75	3.7	68.2	0	....	94.4	0.72	39	28.0	Shapeless
particle size,	150	3.0	68.2	0	....	76.6	0.89	50	21.3	Partly held shape
1.125 inches	225	2.8	68.2	0	....	71.5	0.95	53	23.8	Partly held shape
	300	2.7	68.2	0	....	68.9	0.99	55	27.8	Held shape, very distinct particles
	450	2.6	65.7	2.5	3.7	66.3	0.99	57	34.0	Held shape, very distinct particles
	600	2.4	64.4	3.8	5.6	61.2	1.05	60	37.3	Held shape when lifted, distinct particles
No. 624;	0	6.0	68.0	0	....	153.0	0.44	0	25.4	.....
fat, 40 per cent;	75	3.3	68.0	0	....	84.2	0.81	45	30.3	Held shape loosely
particle size,	150	3.0	68.0	0	....	76.6	0.89	50	31.0	Held shape loosely
0.25 inch	225	2.9	68.0	0	....	74.0	0.92	52	29.8	Held shape when lifted
	300	2.7	68.0	0	....	68.9	0.99	55	29.0	Firm cake, distinct particles
	450	2.6	66.9	1.1	1.6	66.3	1.01	57	33.5	Compact, firm cake with smooth edges
	600	2.5	64.5	3.5	5.2	63.8	1.01	58	40.3	Very firm cake, particles much less distinct
No. 638;	0	6.0	68.3	0	....	153.0	0.45	0	11.7	.....
fat, 35 per cent;	75	3.7	68.3	0	....	94.4	0.72	39	12.3	Shapeless
particle size,	150	3.1	68.3	0	....	79.1	0.86	48	15.3	Loose cake
1.125 inches	225	2.7	66.2	2.1	3.1	68.9	0.96	55	26.3	Loose cake
	300	2.5	63.2	5.1	7.5	63.8	0.99	58	26.8	Irregular cake, fairly firm
	450	2.4	61.3	7.0	10.3	61.2	1.00	60	36.3	Fairly firm cake, distinct particles
	600	2.2	55.9	12.4	18.2	56.1	1.00	63	37.5	Held shape when lifted, distinct particles

[1]Meat dehydrated by the drum-cabinet process and compressed at approximately 25.6°C.(78°F.).[2]Water retained per 100 grams dehydrated meat.

sample was any fat expressed and in that instance only at 450 and 600 pounds' pressure. Under the latter pressure the decrease was 4.8 per cent. This may be compared with 18.2 per cent when the pressing was done at 78°F. At 38°F. the pressures required by Samples 674, 692, 624, and 638 to produce cakes that would hold their form when lifted in the hand were 225, 225, 75, and 150 pounds, respectively.

With the increase up to 600 pounds in applied pressure, each of the four samples increased somewhat in its capacity to take up moisture. These increases were considerably less than those occurring at 78°F. The largest occurred with the high fat-large particle sample and the smallest with the low fat-small particle sample. It will be recalled that only from the former did any fat loss occur. Therefore the increases in rehydration capacity of the other three samples are regarded as caused entirely by some disintegration of the meat particles resulting from the compression. Both disintegration and fat loss, apparently, were factors in the case of the one sample.

The results in general indicate that 1.00 is a desirable level of density for compressed, dehydrated meat. Based on the data obtained, estimates were made of the pressures necessary to attain that density in the three types of vacuum-rotary product at 78°F. and the four types of drum-cabinet product at 78 and 38° F. (Table 3).

TABLE 3

Estimated Pressures Required to Produce a Density of 1.00 in Samples of Dehydrated Meat

Process represented and characteristics of sample	Density of dehydrated meat (uncompressed)	Estimated pressure required to produce a density of 1.00	
		At 25.6°C. (78°F.)	At 3.3°C. (38°F.)
Vacuum-rotary process		*lb./sq. in.*	*lb./sq. in.*
Fat 27 per cent, particle size 0.25 inch	.60	265	.....
Fat 36 per cent, particle size 0.25 inch	.85	50	.....
Fat 38 per cent, particle size 1.125 inches	.60	150	.....
Drum-cabinet process			
Fat 28 per cent, particle size 0.25 inch	.43	400	550
Fat 26 per cent, particle size 1.125 inches	.45	500	600
Fat 40 per cent, particle size 0.25 inch	.44	375	475
Fat 35 per cent, particle size 1.125 inches	.45	450	500

SUMMARY AND CONCLUSIONS

The compressibility of dehydrated pork representing two different processes was studied. Uncompressed, the samples varied widely in density, reflecting differences in fat content and particle size and shape. These factors and temperature of sample were taken into account. Results from compression involved changes in volume, density, texture, fat content, and rehydration capacity.

A high-fat, vacuum-rotary product, compressed at 78°F., had greater density at all pressures than a low-fat product and attained maximum density at much the lower pressure. No fat was pressed from the low-fat

[1]Meat dehydrated by the drum-cabinet process and compressed at approximately 25.6°C. (78°F.). [2]Water retained per 100 grams dehydrated meat.

sample up to at least 600 pounds but the loss was appreciable from the fatter product at 75 pounds. The lower original density of the former resulted in greater reduction in volume at a given pressure.

At all pressures there was greater density in the product of the smaller particle size. However, fat losses from that product were greater than from product of large-particle size, when applied pressures were the same. Large size of particle contributed to greater reduction in volume at a given pressure.

The greatest density at a given pressure was attained in the high fat-small particle product.

Although they varied widely in fat content and, as raw material, in particle size, the drum-cabinet samples varied little in density either uncompressed or at any given applied pressure. High-fat samples appeared to attain maximum density at 450 pounds of pressure; low-fat samples became more dense than those of high-fat content but under greater pressure.

Less fat was expressed from samples of small-particle size. Fat content and particle size appeared about equal in their influence on fat loss.

Decrease in volume resulting from a given pressure varied little among the drum-cabinet samples.

Small size of particle and high-fat content contributed to the holding of shape by the meat cake after compression. No great changes appeared in texture owing to pressure up to 600 pounds at 78°F.

Increase in rehydration capacity appeared mainly due to expressing of fat, with crushing of particles a minor factor.

The behavior of the drum-cabinet samples, when compressed at 38°F., differed little from that at 78°F., with respect to changes in density and volume. Most significant probably was the small fat loss. Increases in rehydration capacity were considerably less than those occurring at 78°F.

Fat expressed from dehydrated meat in small proportion would not be lost under commercial conditions if compressing were done directly in the container. The indications are that 1.00 is a desirable density for the compressed product. It is estimated that 50 to 500 pounds of pressure per square inch would be required to attain that density in the different types of product studied at 78°F., whereas 475 to 600 pounds would be necessary for the drum-cabinet products at 38°F.

VITAMIN A RETENTION IN DRIED EGGS AS AFFECTED BY COMPRESSION AND PACKAGING IN TIN CANS[1]

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Recent investigations on the retention of vitamin A in dried eggs during storage have been reported by Klose, Jones, and Fevold (1943), Hauge, Zscheile, Carrick, and Bohren (1944) and Denton, Cabell, Bastron, and Davis (1944). These workers are in agreement that the rate of destruction of vitamin A in loose-packed dried eggs during storage is closely correlated with temperature.

Most dried eggs prepared for foreign consumption have been packed in barrels with double paper liners or in small breather cartons. In order to conserve shipping space, interest was developed in the compression of dried eggs into brick-shaped packages. There was the further possibility that such compression would improve the retention of the nutrients, especially vitamin A, by reduction of available air in the container and surface area subject to oxidation. No data was available to support this theory, although Klose, *et al.* (1943) mentioned preliminary results which indicated that compression of dried eggs before packaging retarded destruction of vitamin A.

Large quantities of dried eggs have been packed in tin cans for army consumption on the assumption that the limited amount of air and moisture would retard the destruction of nutrients. That such packaging may be beneficial in the preservation of vitamin A is indicated by the data of Klose *et al.* (1943).

The object of this investigation was to determine the value of compression and packaging in tin cans for the preservation of vitamin A in dried eggs during storage.

EXPERIMENTAL PROCEDURE

Fresh midwestern eggs were spray-dried in a cyclone-type dehydrator to a moisture content of less than five per cent. Portions of these eggs were packed as follows: (1) loose in sealed tin cans; (2) loose in waxed fiber cartons; and (3) compressed in waxed fiber cartons. The storage chambers were held at 0, 10, 20, and 37.5°C. (32, 50, 68, and 100°F.). After six months' storage the samples were removed and stored at —18°C. (—0.4°F.) during the period of biological assay. The vitamin A values were determined by the usual rat-growth method, using U.S.P. reference oil diluted with Wesson oil as a standard. Approximately 50 rats were used in assaying each sample; results are given (Table 1).

DISCUSSION

The data indicate that some variability existed between individual packages. This is particularly evident in the sealed cans. The initial

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values of the eggs subjected to the different treatments assayed 59, 62, and 62 I.U. of vitamin A per gram for the loose, compressed, and canned eggs, respectively. This suggests little, if any, difference in the initial potency of the dried eggs after packaging.

Compressing eggs prior to packaging appears to have no beneficial effect on the retention of vitamin A during storage. The eggs which were sealed in tin cans retained their potency as well at 37.5°C. as at 0°C. The beneficial effect of this treatment is most noticeable at the high temperatures of storage. Similar effects of storing dried eggs in sealed tin cans were observed by Klose *et al.* (1943) after nine months' storage at 37°C. (98.6°F.), although the beneficial effects of such packing were not as marked as those reported here.

TABLE 1

Vitamin A in Dried Eggs After Six Months' Storage

Temperature	Loose pack		Compressed		Sealed cans	
	Vitamin A	Retention	Vitamin A	Retention	Vitamin A	Retention
	I.U./gm.	*pct.*	*I.U./gm.*	*pct.*	*I.U./gm.*	*pct.*
Initial (av.)	61	100	61	100	61	100
0°C.(32°F.)	49	80	43	71	44	72
10°C.(50°F.)	46	74	40	66	47	77
20°C.(68°F.)	42	69	38	62	43	70
37.5°C.(100°F.)	18	30	18	30	46	75

The percentage retention of vitamin A in all these samples stored at the various temperatures was consistently higher than the values reported by Klose *et al.* (1943) for their samples but were in good agreement with the values previously reported by Hauge *et al.* (1944) from this laboratory. This suggests that such variations may be due to effects produced by differences in the rations of the hens producing the eggs rather than effects of minor differences in the dehydration processes.

SUMMARY

Dried eggs stored either loose or compressed in waxed fiber cartons for six months at 20°C., or lower, retained 62 per cent or more of their initial vitamin A. At 37°C. they retained only 30 per cent. Compressing the dried-egg powder prior to packaging failed to have any beneficial effect on the retention of vitamin A during storage. Eggs which had been sealed in tin cans retained about 75 per cent of their potency regardless of storage temperatures.

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AN INVESTIGATION OF THE CHARACTERS OF DRIED WHOLE EGG DETERMINING BAKING QUALITY FOR CAKES OTHER THAN SPONGE CAKES

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For the preparation of first-quality sponge cakes the most essential property of egg is its aerating power. Samples of dried egg differ greatly in aerating power, but it has already been shown that the value of a sample for sponge cakes can be easily determined, without recourse to test bakes, by means of a laboratory beating test—Bennion, Hawthorne, and Bate-Smith (1942); Hawthorne and Bennion (1942). At the time the present investigation was undertaken there was no corresponding laboratory test of the baking quality of a sample of dried egg for cakes other than sponge cakes. The work described in this paper was, therefore, undertaken in an attempt to discover what properties contribute to the value of dried egg in such cakes.

In other cakes aeration is, at least in part, achieved by creaming the fat and sugar and by the addition of chemical aerating agents and it might be expected, therefore, that the aerating power of the egg would be of less importance than in sponge cakes. Consideration of the probable functions of egg in ordinary cakes suggests that its value would be determined by the amount of soluble, undenatured, heat-coagulable protein it contains; hence it might be expected that the solubility of a sample of dried egg would be a measure of the baking quality of the samples for ordinary cakes. Attention has primarily been directed in this investigation, therefore, to the correlation between solubility and baking quality but samples differing widely in aerating power have been included in order to determine to what extent independent variation in this property affects the quality of the cakes. The effects of variation in flavor of the egg on cake quality have also been noted.

MATERIAL AND METHODS

With one exception all the samples were spray-dried. They were chosen to show as wide a variation as possible in respect to such properties as solubility, aerating power, and flavor and represented a wide variation in types of spray-dried egg. Some samples were experimental material; a brief description of these is given (Table 1). The remaining samples were drawn from material imported for general distribution.

The laboratory analyses were carried out partly at the Low Temperature Research Station, Cambridge, and partly in the laboratories of the

British Association of Research for the Cocoa, Chocolate, Sugar Confectionery and Jam Trades. The baking tests were made by three commercial bakeries (I, II, III), and by the Research Association.

TABLE 1

Description of Samples of Dried Egg

Sample	Description
C, K	Experimental shipment from U.S.A.
D	Experimental shipment from Australia, prepared from cold-stored eggs.
G	Samples of abnormally high moisture content (8.3 and 11%, respectively) specially prepared in a centrifugal atomizer-type dryer.
H, I, M	Experimental shipment from Canada.
6	Experimental sample of roller-dried egg. Completely insoluble.
9	15 lb. sucrose dissolved in 100 lb. egg pulp and mixture dried in a centrifugal atomizer-type dryer.

1. Analytical Methods:

Details of the L.T.R.S. methods of estimating solubility in KCl, beating power, and flavor have already been published—Bate-Smith, Brooks, and Hawthorne (1943). Solubility was also determined by the refractometric method of Haenni by Hawthorne (1944). The Research Association methods are briefly as follows:

Solubility. Modified Haenni method using water instead of five per cent sodium chloride solution.

Insoluble material. One gram of the sample reconstituted with 10 ml. of distilled water and the solution warmed to 50°C.(122°F.) for 20 minutes. The insoluble material was centrifuged down, transferred to a filter, and weighed.

Jelly test. Fifteen per cent solution in water. Allowed to stand 24 hours at 5°C.(41°F.), aerated under vacuum, then heated in steam for 75 minutes. Cooled in water and left 24 hours at 5°C. Gel strength measured in jelly tester and expressed as rigidity, Campbell (1938).

Beating test. One hundred ml. of a 25-per cent solution in water beaten with a mechanical whisk for 10 minutes at 20°C.(68°F.) and the increase in volume (in ml.) measured.

Fluorescence. The egg powder exposed in the dry state to ultraviolet light. Fluorescence increases with increasing age and decreasing quality; 4 represents no fluorescence, 3 and 2 increasing fluorescence, 1 very bright fluorescence.

2. Baking Tests:

Recipes. Madeira cakes were chosen as being typical of the class of goods in question. The recipes used are shown (Table 2); the columns (a) and (b) refer to the two parts of the investigation as described below.

In all cases in which the sugar-dried egg (Sample 9) was used the recipe was adjusted to allow for the sugar content of the sample.

TABLE 2

Variations in Recipes for Madeira Cakes

Ingredients	I		II		III	Research Assoc.
	(a)	(b)	(a)	(b)	(b)	(b)
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Flour	29.3	31.7	33.2	34.1	29.9	33.1[1]
Corn flour	.....	.....	.....	.....	0.5	.....
Sugar	23.4	19.6	21.6	22.2	23.9	25.5
Shortening	23.4	19.6	14.9	15.4	18.0	15.3
Dried egg }	5.9	7.2	4.2	3.8	6.7	4.1
and water }	17.6	21.5	10.8	11.5	20.2	10.2
Milk	.....	.....	14.1	.....	.....	11.2[2]
Water	.....	.....	.....	11.9	.....	.....
Salt	.....	.....	0.5	0.5	0.3	0.5
Baking powder	0.4	0.4	0.5	0.5	0.6	0.05[3]
Method	Flour batter		Sugar batter		Sugar batter	Sugar batter
Oven temperature	380°F.		400°F.		360°F.	370°F.
Baking time	60 min.		40 min.		.....	50 min.

[1] Self rising. [2] Dried milk, reconstituted. [3] Sodium bicarbonate.

3. METHOD OF MEASURING CAKE VOLUMES:

The cakes were halved, a section photographed, the negative placed in an enlarger, and an image thrown onto squared paper so that a scale included in the photograph occupied a definite number of divisions on the squared paper. An outline of cake was then drawn, following as closely as possible the cut edge of the cake. For this purpose of calculating the volume, the cake was imagined as consisting of a number of concentric, annular, cylindrical shapes. The base of each of these annular cylinders is an annular ring whose area can be calculated from the formula:

$$\text{Base area} = \pi r_1^2 - \pi r_2^2 = \pi(r_1+r_2)(r_1-r_2)$$

where r_2 and r_1 are the inner and outer radii of the ring.

The heights corresponding to the different annular cylinders were read from the outline and the volume calculated.

Allowance was made for the space through which the top edge of the cake shrank on cooling.

RESULTS

The investigation was carried out in two parts. In the first test 11 samples (A to M) were used, representing the widest variation in quality of spray-dried egg which could be obtained at that time. Reference to Table 3, in which the results of the laboratory analytical tests are given, will show that the samples showed wide differences in solubility, aerating power, and flavor. In spite of this there was little difference between the cakes made with the samples [the baking recipes are shown under (a) in Table 2], and all the cakes were judged to be of good commercial quality.

The conclusion reached as a result of this test, therefore, was that within wide limits, the quality of dried egg was of no demonstrable significance in the baking of ordinary cakes.

Shortly after the completion of this test Stuart, Grewe, and Dicks (1942) published a paper in which they stated that they had found a good correlation between the quality of pound cakes and the solubility of the dried egg used in their preparation. It was thought desirable in view of this paper to repeat the work, varying the properties of the egg more widely than before.

TABLE 3

Results of Laboratory Analytical Tests

Sample	Solubility in KCl (c)[1]	Haenni value		Insoluble (d)	Jelly test (d)	Beating test		Fluorescence (d)	Flavor score (c)
		(c)	(d)[1]			40°C. (c)	20°C. (d)		
First Test									
	pct.			*pct.*					
G	98	23.8	25.8	0.15	29.2	410	96	4	7
I	95	24.5	26.0	1.66	23.9	440	100	3	7
C	95	24.6	25.9	0.91	27.9	190	18	3	7
H	94	24.5	25.8	1.45	23.4	345	88	3	7
M	91	22.8	25.5	1.72	22.4	200	76	2	6½
K	88	22.5	23.9	6.00	23.1	270	48	4	7
A	85	19.1	25.8	0.55	26.3	290	60	3	6
F	83	19.7	24.1	8.17	24.8	250	66	4	6½
B	80	16.5	21.1	4.65	18.6	140	49	3	4½
D	76	.....	23.0	6.28	25.0	165	30	2	5½
E	63	11.2	10.3	42.4	12.2	100	0	1	3
Second Test									
9	99	.....	24.9	.....	.....	.....	390	3	....
7	97	24.0	24.3	.....	29.0	340	70	4	6½
5	94	25.0	25.1	0.8	29.0	225	20	4	7
2	84	20.0	23.3	0.7	25.8	290	83	4	6
3	71	13.5	14.1	.....	25.2	160	84	3	5
8	64	12.0	12.8	.....	19.0	140	20	3	4
10	....	.....	11.5	.....	.....	.....	31	1	....
1	50	10.0	10.0	.....	13.5	100	10	2	3½
4	43	9.5	9.2	.....	11.9	100	16	2	1½
6	4	.....	2.9	79.9	nil	100	nil	1	....

[1](c) L.T.R.S. methods; (d) Research Association methods.

For this second test Madeira cakes were again chosen as the test cake; the recipes [shown under (b) in Table 2] were similar to those used in the first test. Ten samples (1 to 10) of dried egg were used, the analyses of which are shown (Table 3). Three of the samples were of poorer quality than any of the material available for the first test; Samples 1 and 4 were both very poor commercial specimens of spray-dried egg; while Sample 6 was a specimen of roller-dried egg which had been almost completely denatured during the drying process and had a solubility of only four per cent. Samples 1 and 10 had both been the subject of complaints by commercial bakers. In addition to these very poor specimens Sample 9, a specimen of egg dried with sugar, was included. It has already been shown by Brooks and Hawthorne (1943) that such material has excep-

tional aerating power and it was hoped that by inclusion of this sample any effect of the aerating power of the egg in determining the quality of ordinary cakes could be conclusively demonstrated.

In this second test more clear-cut results were obtained than in the first part of the investigation. The placing of the samples by the individual collaborators as the result of their baking tests is shown (Table 4). The baking quality of the samples was judged by the volume, appearance, and texture of the cake and the behavior of the batter in the oven.

TABLE 4

Rating of Cake Samples by Individual Collaborators

Collaborators	Good	Poor
Research Association	7 5 2 (3 9)	(1 8) 4 6
Bakery I	(7 9) 5 3 (1	2 4 8 6)
Bakery II	9 7 5 2 8	3 1 4 6
Bakery III	7 (5 9) 2 8	3 4 1 6
Solubility	9 7 5 2 3	8 1 4 6

The general comments showed that the samples fell into three groups: Samples 9, 7, and 5 gave excellent cakes; 1, 4, and 6 very poor cakes; while the cakes made with Samples 2, 3, and 8 were variously described as fair to good. It is clear, therefore, that in this test a definite correlation was found between the quality of the cakes and the quality of the samples of dried egg as shown by the laboratory tests. In particular, the correlation with solubility is very close.

The volume of the cakes made in the laboratories of the Research Association are shown (Table 5). From the data in Tables 3 and 5 the correlation coefficients between solubility and cake volume and between aerating power and cake volume have been calculated. The coefficients are solubility/volume 0.85, aerating power/volume 0.31. Since for 10 experiments a coefficient of at least 0.63 is required to indicate a definite correlation between either of the two pairs of variables, it is safe to conclude that solubility is the important characteristic as regards baking quality for ordinary cakes and that aerating power is, at the most, of minor importance.

TABLE 5

Volume of Cakes Made in Research Association Laboratories

Sample	Cake volume	Sample	Cake volume
	c.c.		*c.c.*
1	1154	6	744
2	1224	7	1296
3	1198	8	1146
4	1103	9	1277
5	1288	10	1188

The results obtained with Sample 9 furnish a good illustration of this point. In general it is true that a sample of spray-dried egg of good aerating power is a good sample in all other respects, though the converse

is not necessarily true (cf. Sample C which had good solubility, flavor, etc., but was almost entirely lacking in aerating power, even when whisked at 40°C.). Using ordinary spray-dried egg, therefore, it is difficult to separate the effects of solubility and aerating power in the preparation of cakes. It has already been shown, however, that egg dried with sugar has much better aerating power than ordinary spray-dried egg, equal in fact to fresh or frozen egg according to Brooks and Hawthorne (1943). In this test the sugar-dried egg gave results with Madeira cakes little, if any, better than samples of equal solubility but greatly inferior aerating power. The particular merit of sugar-dried egg for ordinary confectionery work, therefore, lies not in its excellent aerating power, but in the fact that owing to its better keeping qualities it will retain its high solubility under conditions which would result in excessive deterioration of ordinary spray-dried egg, Brooks and Hawthorne (1943).

Experiments made with a view to discovering any connection between staling of the cakes, either in terms of moisture loss or loss of palatability (staling), and egg quality failed to give any clear-cut decision. What correlation exists between the solubility of the dried egg and staling suggests that cakes made with the more soluble egg, being of more open texture, tend to stale rather more rapidly than denser cakes made with less soluble dried egg. There is certainly no evidence to suggest that the greater solubility of the better samples of dried egg assists the retention of moisture in the cake.

ADJUSTMENT OF RECIPE TO CORRESPOND WITH SOLUBILITY OF DRIED EGG

In the above tests the samples of egg were used in standard recipes and, therefore, although the total amount of egg in each test was constant, the amount of soluble egg varied. The results show that there is a correlation between the solubility of the samples and the quality of the cakes, that is, that the amount of soluble egg is the chief factor in determining cake quality. It might be expected, therefore, that with samples of low solubility, which give poor results in standard recipes, an improvement could be achieved by increasing the total amount of egg powder in the recipe. Further experiments in the laboratories of the Research Association have shown this to be the case.

In these experiments cakes containing different proportions of egg were baked, the fat content and total weight of the batter being kept constant by adjustment of the weight of margarine and flour; typical results are shown (Table 6).

It is clear that these experiments again show a close correlation between the amount of soluble egg and the quality of the cake. They also demonstrate the improvement which can be achieved in the case of samples of low solubility by suitable adjustment of the recipe. It may be mentioned, however, that while increasing the amount of egg gave a cake of greater volume and, in general, of better over-all quality, with a number of samples of egg the use of large amounts gave cakes of tough texture.

A further point in connection with the results of these tests may be mentioned. In both tests the differences between the cakes were less

pronounced when the flour-batter technique was used than when the sugar-batter procedure was employed. We are informed that this is generally recognized by practical bakers. Nevertheless, whichever technique is followed it appears that dried egg of high solubility gives better results than egg of low solubility.

TABLE 6

Results of Cake Tests Using Different Proportions of Dried Egg

Sample	Egg powder	Soluble egg[1]	Volume of cake	Description of cake	
				Crust	Crumb
	pct.	*pct.*	*c.c.*		
4	4.1	1.5	1103	Flat	Dense
4	11.1	4.1	1280	Fairly bold, cracked	Light, rather hard
1	4.1	1.6	1154	Flat	Dense
1	10.2	4.1	1225	Fairly bold, cracked	Light, rather hard
K	4.1	2.0	1188	Flat, soft	Light, open
K	8.7	4.0	1220	Flat, soft, not cracked	Light, feathery
5	4.1	4.1	1288	Bold, cracked	Light, open
5	8.2	8.2	1434	Very bold, cracked	Very light, rather hard
7	4.1	4.0	1296	Bold, cracked	Light, feathery
7	1.6	1.6	941	Flat	Dense
9	4.1	4.1	1277	Bold, cracked	Light, open
9	1.6	1.6	957	Flat	Dense

[1] Calculated from refractive index measurements.

While the quality of the cake made with any particular specimen of dried egg appears to depend, to some extent, on the baking technique employed, the results of this investigation show that the solubility of the sample is a good measure of the baking quality for cakes of the Madeira type, while the aerating power is of little value as an index of baking quality. Further experiments have shown that the presence of fruit in the cake does not affect this conclusion.

SUMMARY

Baking tests (Madeira cakes) have shown that there is a correlation between the baking quality and the solubility of samples of dried whole egg. The aerating power of the egg appears to be of little importance in the preparation of this type of cake.

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IMPROVEMENT IN DIGESTIBILITY OF NAVY-BEAN STARCH

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In an earlier note attention was called to a means of compensating for digestive inaccessibility of the starch of navy beans, Bowman (1944). It was suggested that the well-known action of acid upon raw starch might be applied. In view of a certain interest which has been shown in these observations and their application, somewhat greater detail may be desirable.

A review of the literature indicates that in 1917 Pauletig (1917) called attention to the greater ease with which cereal starches are digested as compared with the starches of legumes. For these observations a paste was made from the prepared starches. It was pointed out that bean starch is particularly resistant although there is some question regarding the type of beans used and the manner in which the starch was prepared.

In the present investigation particular attention was paid to the *in vitro* digestibility of the carbohydrate fraction of common commercial navy beans. The manipulation of the control preparations was limited to that involved in soaking, heating, and mashing the beans in order that the substrate might resemble that of consumed beans. The digestive availability of the carbohydrate fraction was compared with that of similarly treated potatoes because of the wide acceptance of the latter as a common and good source of dietary starch.

METHODS

In general the procedure followed consisted of soaking dried navy beans for at least 12 hours in water or in dilute acid. The beans were then heated for varying periods and thoroughly and uniformly mashed. The reducing sugar concentration resulting from the digestive action of pancreatic amylase was employed as a means of following the degree of starch hydrolysis. The digestive substrate was prepared by suspending the mashed material in sufficient water to provide a one-per cent concentration of carbohydrate. In order to simulate the substrate which might be available to the actual digestive processes of the body no attempt was made to separate the starch or other carbohydrates from the cooked and mashed substance. In the amylolytic digestion one ml. of 0.8 M phosphate buffer at pH 7, one ml. of 10 per cent sodium chloride and two ml. of 0.5 per cent commercial pancreatin were added to 25 ml. of the suspension. Before incubating at 38°C.(100.4°F.) the pH of the digestion mixture was checked by means of a glass electrode pH meter. The relative concentrations of reducing substances were expressed in terms of the reducing power of glucose and were estimated by employing Folin and Wu's (1920) alkaline copper reagent and by Shaffer and Somogyi's (1933) method of

determining reducing sugar concentrations. The results of the two methods were parallel and led to the same relative conclusions throughout, although values obtained with the former method were somewhat higher than with the latter.

In each experimental series a control consisting of one per cent soluble starch, prepared according to Lintner, was used simultaneously for purposes of comparison.

TABLE 1

Relative Digestibility of Bean and Potato Carbohydrates

Material	Method of heating	Reducing material expressed as maltose derived from one per cent starch	
		30 minutes' digestion	60 minutes' digestion
		pct.	*pct.*
Soluble starch	Boiled 5 min.	.550	.610
Potatoes No. 1	Boiled 1 hr.	.250	.500
Potatoes No. 2	Boiled 1 hr.	.140	.430
Potatoes No. 3	Boiled 1 hr.	.130	.410
Potatoes No. 4	Boiled 1 hr.	.180	.470
Potatoes No. 5	Boiled 1 hr.	.180	.470
Potatoes No. 6	Boiling water bath 1 hr.	.410	.540
Whole beans No. 1	Boiled 1 hr.	.049	.054
Whole beans No. 2	Boiled 1 hr.	.054	.055
Whole beans No. 3	Boiled 1 hr.	.054	.054
Whole beans No. 4	Boiled 1 hr.	.036	.035
Whole beans No. 5	Boiled 1 hr.	.050	.050
Whole beans No. 6	Boiled 1 hr.	.035	.055
Whole beans No. 6	Boiling water bath 2 hr.	.016	.052
Whole beans No. 6	Boiling water bath 4 hr.	.018	.055
Whole beans No. 6	Boiling water bath 8 hr.	.035	.050
Whole beans No. 6	Boiling water bath 24 hr.	.070	.072

RESULTS

The slower digestion of the navy-bean starch as compared with that of potatoes is readily apparent when both foods are heated in a similar manner. The rather striking difference may be observed from the data (Table 1) which are typical of many similar experiments. The white potatoes referred to in this table were obtained from five different sources. They were cut into pieces the approximate size of navy beans and boiled or heated to 100°C.(212°F.) in water for one hour. It may be observed that the availability of the starch of such potatoes compares much more favorably with soluble starch than does the bean starch. In fact beans which have been soaked and heated at 100°C. for as long as 24 hours are still relatively resistant to amylolytic hydrolysis.

Various factors were investigated as possible causes of the difficulty with which the starch of these beans undergoes enzymatic digestion. The fact that finely grinding the beans in the dry state leads to a considerable improvement in carbohydrate availability suggests that structural factors are important. Beans so treated were ground to the consistency of a fine

meal in a hand corn mill and were then soaked and heated in a manner similar to that described for treatment of whole beans; typical results are given (Table 2). The marked increase in the accessibility of the starch of beans which have been ground in the dry state does not appear to result from a difference in the size of the particles at the time of amylolytic digestion since all preparations were quite similar at this point.

TABLE 2

Digestibility of Carbohydrates of Beans Finely Ground in Dry State

Material	Method of heating	Reducing material expressed as maltose derived from one per cent starch	
		30 minutes' digestion	60 minutes' digestion
		pct.	*pct.*
Soluble starch	Boiled 5 min.	.55	.61
Beans No. 1	Boiling water bath 2 hr.	.29	.30
Beans No. 2	Boiling water bath 2 hr.	.25	.26
Beans No. 3	Boiling water bath 2 hr.	.24	.27
Beans No. 4	Boiling water bath 2 hr.	.25	.31
Beans No. 5	Boiling water bath 2 hr.	.27	.28

This favorable influence of grinding the beans in the dry state is considerably greater in magnitude, though apparently similar in principle, to that observed by Peterson and Churchill (1921) upon grinding to an impalpable powder navy-bean starch which had been ground originally in an ordinary feed mill. The increase in the digestible starch in the finely ground preparation was reported as 10 to 12 per cent.

In order to decrease the digestive resistance of the bean starch without seriously altering the form or taste of the beans various procedures were employed and it is believed that one of the most practical methods used involves the well-known hydrolytic action of acid upon raw starch. Through the employment of a preliminary acid treatment varying degrees of improvement in the digestibility of the bean starch can be obtained by varying the heating period before or after acid treatment and, of course, by varying the concentration of the acid. The influence of varying concentrations of hydrochloric acid is shown by the typical findings represented (Table 3). The acid-treated beans referred to in this particular series were soaked for 48 hours in the acid solution indicated, after which the acid was decanted, water added, and the beans were maintained at 100°C. for 40 minutes. After thoroughly mashing the beans the reaction of the suspension was adjusted to pH 7 with 10 per cent sodium hydroxide and the availability of the starch was estimated.

It is apparent that the final reducing sugars derived from the beans can be made to compare favorably with those resulting from the hydrolysis of soluble starch if sufficient acid is employed. However, extensive predigestion is usually associated with partial hydrolysis of the starch and a tendency toward the destruction of the form of the beans. One-tenth normal hydrochloric acid was used most frequently with results consistently quite similar to that indicated. In such preparations the change in general appearance is not significant.

Considerable variation in the resistance of different lots of beans to the influence of the acid was observed. In some instances it was necessary to increase considerably the period of heating or the acid concentration in order to attain the digestibility already described and observed in most of the lots employed. In such cases it was found to be even more advantageous to infiltrate the beans with the acid, boiling them in the presence of the acid after 24 hours and again at 48 hours. In this manner it was found that the two 30-minute periods of heating were sufficient to overcome the unusual resistance encountered in certain lots, imparting digestibility quite comparable to that indicated for 0.1 N acid (Table 3). It would appear that for practical purposes such a procedure might be advisable routinely.

TABLE 3

Influence of Varying Concentrations of Hydrochloric Acid

Concentration of acid	Reducing material expressed as maltose derived from one per cent starch		
	Before amylolytic digestion	After 30 minutes' digestion	After 60 minutes' digestion
	pct.	*pct.*	*pct.*
0.1 N	.055	.25	.27
0.5 N	.072	.27	.29
1.0 N	.23	.41	.43
2.0 N	.32	.54	.54
5.0 N	.50	.58	.60

As might be expected autoclaving serves a purpose similar to the acid treatment of the starch. However, although various lots differ, as long as 90 minutes at 15 pounds pressure may be required to provide the same degree of digestibility as boiling in 0.1 N acid for 50 minutes. Therefore, it would appear that the latter method might be more generally applicable and at the same time serve to minimize the destruction of heat-labile vitamins which are particularly unstable in neutral or alkaline solutions.

Neutralization of the beans may be accomplished, of course, by dilute solutions of sodium hydroxide or sodium bicarbonate allowing time for infiltration with periodic checking. By carefully standardizing the details, the required checking can be minimized.

DISCUSSION

Since it is apparently necessary to add sufficient acid to partially hydrolyze bean starch prior to enzymatic digestion in order to produce a final total reducing sugar concentration comparable to that derived from simple amylolytic digestion of cooked potato starch, factors not yet taken into account also may be involved.

Although dietary significance may not be outstanding, further work on amylase retarding characteristics which do not involve structural factors will be described subsequently.

However, since starch which is only partially available to digestive processes might be considered, at least in part, as being comparable to

raw starch it is of interest to recall the observation of Beazell, Schmidt, and Ivy (1939). These investigators have found that the ingestion of a diet consisting of 63 per cent raw potato starch leads to the excretion of a considerable amount of the starch in the feces, usually associated with marked flattulence apparently resulting from bacterial fermentation of a part of the starch. They suggest that fermentation accounts for an important part of the raw potato starch that is decomposed in the alimentary tract, indicating that the coefficient of digestibility does not always apply to useful digestion alone. In view of the present findings it is quite possible that the *in vivo* digestion of the starch of navy beans may present an analogous situation.

Similarly, it is possible that more emphasis might well be given to observations such as those of Hurst and Knott (1930) who have referred to a type of intestinal carbohydrate dyspepsia in humans characterized by much flattulence, abdominal dyspepsia, and diarrhea associated with the appearance of unruptured starch granules in the feces. This is attributed to inadequate amylolytic digestion in the small intestine with hydrolysis and bacterial fermentation in the large intestine.

While the improvement in the availability of bean starch as described here is essentially a procedure requiring laboratory control, the collection of additional data which is in progress may further aid in establishing a simple standardized procedure generally applicable in all cases. Since the favorable action of dilute acids is not limited to the present instance or to carbohydrates, such additional observations include practical aspects of the application of this well-known principle to other foods.

SUMMARY

The starch of cooked navy beans is relatively resistant to digestion by pancreatic amylase.

This unfavorable digestibility can be considerably decreased by preliminary acid treatment without destroying the attractiveness of the beans as a food.

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VEGETABLE CROPS IN RELATION TO SOIL FERTILITY. III. OXALATE CONTENT AND NITROGEN FERTILIZATION[1]

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Green leafy vegetables may be one of our richest sources of dietary calcium. Although these crops generally make adequate growth over a wide range of soil differences, the fertility level may presage a wide range in their mineral composition. Of late, the relative availability or digestibility of organo-calcium and other mineral-organic complexes in many of these vegetables has been the subject of numerous investigations, such as those of Tisdall and Drake (1938), Spiers (1939), and Kohman (1939). A summary of this research, which has involved feeding tests conducted on both man and smaller animals, shows rather conclusively that many common leafy vegetables, including spinach, Swiss chard, beet greens, and New Zealand spinach, may be worthless as contributors of calcium and magnesium and in addition, if consumed in moderate quantities, may render unavailable a large percentage of the calcium normally furnished in the diet by other foods. This undesirable feature is attributed to the large amounts of oxalic acid which probably combine not only with calcium in the plant but also with the magnesium to form insoluble oxalates, Pierce and Appleman (1943), making these two essential elements from the soil valueless so far as human nutrition is concerned.

Since these oxalate-containing foods now play a prominent, though perhaps not a desirable, role in the human diet, an analysis of plants for the chemically equivalent relationships between their oxalate contents on the one hand, and their concentrations of calcium and magnesium on the other, was deemed essential. Moreover, the wide fluctuations in oxalic acid known to occur in a given crop of the same variety, as indicated by Kohman (1939), prompted a further inquiry into possible soil factors, causal of this variation, other than those of calcium and pH already outlined by Schroeder and Albrecht (1942). The following investigation represents an analysis and study of the oxalate and mineral contents of Swiss chard (variety Lucullus), which was subjected to 16 levels of soil nitrogen and calcium as combinations of four different amounts of each.

EXPERIMENTAL PROCEDURE

Satisfactory control in providing the desired fertility gradients was achieved by using the subsoil of Putnam silt loam as a carrier for exchangeable soil nutrients. This material, with its high content of colloidal acid clay, has the semblance of natural soil and permits the uptake of nutrients by the plant through contact exchange.

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A series of soil treatments was prepared by supplying calcium and nitrogen levels each of 5, 10, 20, and 40 milliequivalents (me.) with all possible (16) combinations of two nutrients. Accordingly, for each level of calcium there were four levels of nitrogen, and for each level of nitrogen there were four of calcium. The calcium and nitrogen were provided by adding variable quantities of calcium acetate and ammonium nitrate, respectively, to the correspondingly required clay aliquots, replacing the adsorbed hydrogen thereon with cationic nutrients. Other materials supplied in constant amounts consisted of 20 me. each of potassium and phosphorus, and six me. each of magnesium and sulfate. The resulting clay samples, almost completely saturated with various ratios of exchangeable calcium and nitrogen, were, by using moisture, blended with pure white quartz sand. Each treatment was replicated 10 times, the plants being grown in one-gallon glazed crocks in which one plant for each container was retained till harvest time. The nutrients and the clay supplied for each plant with the resulting pH are presented (Table 1).

TABLE 1

Nutrients Added to Clay to Provide Variable Calcium and Nitrogen Levels

Treatment	Milliequivalents per plant						Clay per plant	Resulting pH
	Ca	N	P	K	Mg	S		
				Ca Group I[1]				
							gm.	
1	40	40	20	20	6	6	716.6	6.5
2	40	20	20	20	6	6	633.3	6.8
3	40	10	20	20	6	6	591.6	6.8
4	40	5	20	20	6	6	570.8	7.0
				Ca Group II				
5	20	40	20	20	6	6	550.0	6.4
6	20	20	20	20	6	6	466.6	6.6
7	20	10	20	20	6	6	425.0	6.6
8	20	5	20	20	6	6	404.1	6.8
				Ca Group III				
9	10	40	20	20	6	6	466.6	6.4
10	10	20	20	20	6	6	383.3	6.5
11	10	10	20	20	6	6	341.6	6.6
12	10	5	20	20	6	6	320.8	6.6
				Ca Group IV				
13	5	40	20	20	6	6	425.0	6.6
14	5	20	20	20	6	6	341.6	6.4
15	5	10	20	20	6	6	300.0	6.5
16	5	5	20	20	6	6	279.1	6.5

[1] In a like manner four N groups may be arranged, each containing four levels of Ca.

The experiment was conducted under greenhouse conditions during the late fall and early winter 1943-44. All plants were harvested after a growing period of 90 days, at which time they were judged to be in the most desirable eating condition. The tops were cut, weighed, dried at 65°C. (149°F.), and the total dry matter recorded. After being shredded

in a Wiley mill and finely ground in a Merker mill, the dried material was suitable for analyses. The chemical determinations consisted of oxalate, total nitrogen, calcium, magnesium, and phosphorus. All analyses were performed in accordance with regular laboratory methods recognized as official or standard procedures.

RESULTS AND DISCUSSION

The total yields as fresh weights and the results of the chemical analyses in terms of per cent concentrations in the dried material are assembled (Table 2). The treatments are first arranged into calcium groups, of which the four different nitrogen levels are averaged to show the influence of each level of calcium. Secondly, the treatments are regrouped according to the nitrogen applied, and the mean values of the calcium treatments taken so that the average for each nitrogen level could be recorded.

The most striking features evident from the data presented seem to be the following: (1) the magnitude of yield increase was about equal when either nitrogen or calcium was applied in additive quantities; (2) regardless of the calcium supply, the percentage of oxalate in the crop generally increased as more nitrogen was provided; (3) the percentage of total nitrogen in the crop was directly correlated with that of the oxalate and with the nitrogen supplied in the substrate; (4) the percentages of plant calcium were consistently greater as the calcium in the soil was increased, while the magnesium at first varied directly with the increments of both calcium and nitrogen and then showed a decided drop when the highest level of either was reached; and (5) there were marked differences, owing to soil treatment, in the magnesium and phosphorus percentages although the quantities of these nutrients applied to the soil were constant for all groups.

These results are of special significance in that they demonstrate remarkable variability in the important dietary components within the same crop, as a result of growing it on soil media differing in fertility. Differences in fertility not only gave variances in the quantities of the mineral elements moved from the clay-sand mixture into these plants, but it is likely that much of the fluctuation in the concentrations of the oxalate—a synthetic product of the plant's metabolism—may be accounted for by variability in the fertility of the soil.

Oxalate in chemical combination with calcium forms a very insoluble compound. This is likely true also of its union with magnesium. Since some of the leafy vegetable crops have been reported as containing more than sufficient oxalate to precipitate and put the entire supply of these two mineral nutrients into inert forms, so far as ordinary digestive processes are concerned, it seemed essential in these studies to examine to what degree the oxalate was stoichiometrically balanced by the calcium and magnesium in Swiss chard. These relations are best shown by Fig. 1, wherein the probable fate of the total plant oxalate is portrayed.

Several facts are evident. Stoichiometrically, the oxalate content exceeded by several times that of calcium at all fertility levels. In every instance the total oxalate was in excess of the plant calcium and magnesium combined. This left a sizeable surplus of soluble oxalates for possible

TABLE 2

Yield, Oxalate Content, and Mineral Composition of Swiss Chard When Grown Under Variable Levels of Nitrogen and Calcium

Treatment	Variable me. applied		Total yields in fresh weight per 10 plants	Concentrations in per cent of dry weight				
	N	Ca		Oxalate	Total nitrogen	Calcium	Magnesium	Phosphorus
Ca Group I								
1	40	40	634.6	6.63	4.29	1.032	.477	1.33
2	20	40	486.4	5.58	3.16	0.888	.416	1.30
3	10	40	233.3	4.70	2.58	0.886	.534	1.87
4	5	40	179.8	3.93	2.40	0.748	.320	2.67
Average	....	....	383.5	5.21	3.10	0.889	.437	1.79
Ca Group II								
5	40	20	487.3	6.47	4.61	0.784	.734	1.04
6	20	20	693.5	5.57	3.92	0.676	.864	1.10
7	10	20	243.4	4.79	2.63	0.720	.678	1.05
8	5	20	133.8	4.32	2.06	0.776	.542	1.66
Average	....	....	389.5	5.29	3.31	0.739	.705	1.21
Ca Group III								
9	40	10	391.6	5.63	4.57	0.600	.440	1.06
10	20	10	333.8	6.94	3.97	0.776	.720	0.88
11	10	10	215.2	4.38	2.53	0.824	.552	1.41
12	5	10	116.6	3.78	2.43	0.746	.368	2.04
Average	....	....	264.3	5.18	3.38	0.737	.520	1.35
Ca Group IV								
13	40	5	32.0	6.50	4.15	0.488	.380	1.81
14	20	5	212.7	7.34	3.91	0.820	.646	0.85
15	10	5	171.5	5.00	2.77	0.660	.284	1.11
16	5	5	104.6	3.96	2.25	0.552	.390	1.57
Average	....	....	130.2	5.70	3.27	0.630	.425	1.34
N Group I								
1	40	40	634.6	6.63	4.29	1.032	.477	1.33
5	40	20	487.3	6.47	4.61	0.784	.734	1.04
9	40	10	391.6	5.63	4.57	0.600	.440	1.06
13	40	5	32.0	6.50	4.15	0.488	.380	1.81
Average	....	....	386.4	6.31	4.41	0.726	.508	1.31
N Group II								
2	20	40	486.4	5.58	3.16	0.888	.416	1.30
6	20	20	693.5	5.57	3.92	0.676	.864	1.10
10	20	10	333.8	6.94	3.97	0.776	.720	0.88
14	20	5	212.7	7.34	3.91	0.820	.646	0.85
Average	....	....	431.6	6.36	3.74	0.790	.662	1.03
N Group III								
3	10	40	233.3	4.70	2.58	0.886	.534	1.87
7	10	20	243.4	4.79	2.63	0.720	.678	1.05
11	10	10	215.2	4.38	2.53	0.824	.552	1.41
15	10	5	171.5	5.00	2.77	0.660	.284	1.11
Average	....	....	215.9	4.72	2.63	0.773	.512	1.36
N Group IV								
4	5	40	179.8	3.93	2.40	0.748	.320	2.67
8	5	20	133.8	4.32	2.06	0.776	.542	1.66
12	5	10	116.6	3.78	2.43	0.746	.368	2.04
16	5	5	104.6	3.96	2.25	0.552	.390	1.57
Average	....	....	133.7	4.00	2.29	0.706	.405	1.99

combination with calcium derived from other foods in the diet. It is also to be noted that the quantity of oxalate in excess of the plant calcium alone, or of the summation of the calcium and magnesium, was directly correlated with the nitrogen offered in the soil. This excess was particularly marked when the calcium supply was low. The excess soluble oxalates diminished as the nitrogen supply decreased. This was true regardless of the calcium level.

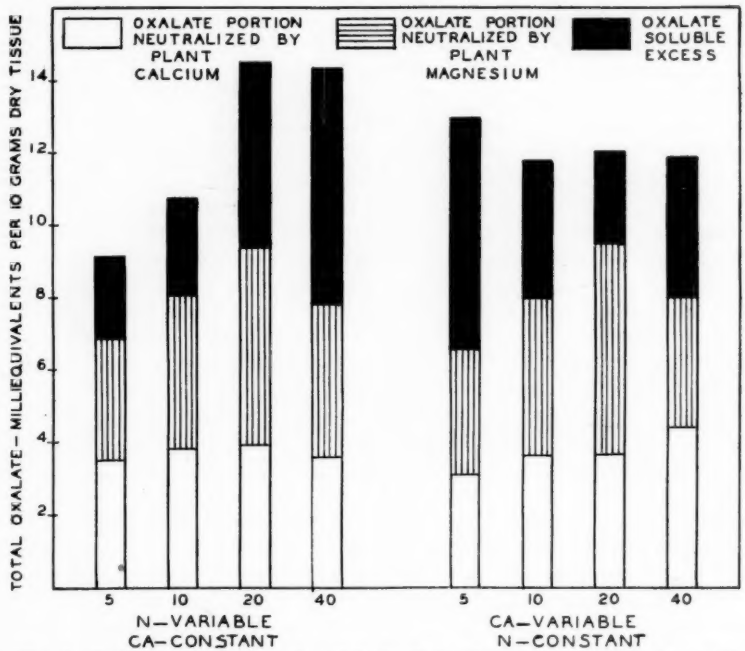

FIG. 1. Probable disposition of oxalate in Swiss chard when grown under variable levels of nitrogen and calcium in the soil.

Though the above relations between plant oxalates and the degree of nitrogen fertilization present a new departure in plant nutrition with respect to the nutritive quality of some leafy vegetables, the basic concept that nitrogen fertilizers may profoundly influence the organic acid composition of other crops was first emphasized by Ruhland and Wetzel (1926). This effect is accentuated if nitrate rather than ammonium nitrogen is used, according to Clark (1936); Wadleigh and Shive (1939); Vickery, Pucher, Wakeman, and Leavenworth (1940); and Blackman and Templeman (1940). Pepkowitz, Gilbert, and Shive (1944) suggest a direct association of oxalic acid formation with nitrate reduction. In the present investigation, ammonium nitrate was used as the nitrogen fertilizer. If nitrate-nitrogen alone had been employed, the results might have proven even more conspicuous.

These data, interpreted in conjunction with those of Schroeder and Albrecht (1942), suggest that soil-fertility practices in the production of Swiss chard and spinach may offer a certain degree of control over the quantities of oxalate produced. One would expect the least disturbance from oxalate when the crops are grown on an acid soil high in exchangeable calcium and low in readily available nitrogen. Wittwer, Schroeder, and Albrecht (1945) have demonstrated that heavy applications of nitrogen as a fertilizer salt seem to lessen their mineral as well as vitamin C contributions in the human diet. With reference to food qualities, it is unfortunate in the production of these vegetables that the yield response is so favorable toward increased nitrogen fertilization, as shown by Schroeder and Wittwer (1944).

Where dietary preferences permit, spinach, Swiss chard, beet greens, and New Zealand spinach may be replaced by other green leafy vegetables known to be free from the harmful effects of oxalate. Such crops include kale, mustard greens, collards, and turnip greens. The initial supply of all basic minerals and vitamins in this latter group of vegetables generally exceeds that of the high oxalate plants, and, containing an insignificant amount of oxalate, they contribute calcium and magnesium in forms that are readily assimilated by the digestive system.

SUMMARY

By a study of the oxalate-calcium and oxalate-magnesium relations in Swiss chard, grown under 16 levels of variable calcium and nitrogen in colloidal clay cultures, it was shown that the concentrations of oxalate in the crops increased as more nitrogen was provided in the substrate. This increase seemed to be independent of the calcium supply.

When the chemically equivalent quantities of oxalate with respect to its possible union with the plant's calcium and magnesium were considered, it was demonstrated that for all fertility levels the total oxalate production was far in excess of its equivalent of the plant's calcium, or even of calcium and magnesium combined. These surpluses of oxalate, beyond precipitation by calcium and magnesium, were progressively of greater magnitude as the nitrogen supply was increased and the calcium decreased.

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RETENTION OF CAROTENE, THIAMIN, RIBOFLAVIN, AND NIACIN DURING LARGE-SCALE FOOD SERVICE OF DEHYDRATED CARROTS[1]

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No reports were found in the literature on the retention of vitamins in large-scale food service of dehydrated carrots. The purpose of this study was to determine the losses of carotene, thiamin, riboflavin, and niacin from commercially dehydrated carrots during quantity cooking by the following methods in amounts to serve 50: (1) simmering on top of the stove, in enough boiling water to cover, for 30 minutes (directions on the can label); (2) in an institution-type steamer for 15 minutes; (3) boiling for 25 minutes on top of the stove, in enough boiling water to cover, in a U. S. Army field range; (4) simmering on top of the stove for 20 minutes, after refreshing for 20 minutes and after heating for 45 minutes to bring to a boil (directions in Swift's Army Cook Book); (5) same as (4) except using carrot bricks and refreshing for 90 minutes before cooking. Because it is often necessary in Army mess halls to keep cooked vegetables at serving temperature for varying periods of time, the carrots cooked by directions in the Swift's Army Cook Book were held for one and one-half and for three hours, after which vitamin losses were determined.

HISTORY OF VEGETABLE

The carrots, dehydrated by the Beechnut Packing Company, were of the Chantenay variety. They were cut in one-fourth-inch cubes, and were steam-blanched previous to dehydration. Raw carrots of the same variety, which had been dehydrated in the same plant two days previous to the dehydration of those used in this study, were analyzed for carotene and ascorbic acid.[2] The raw carrots contained 95 mg. of carotene per 100 gm. on the dry-weight basis. There was no change in value during dehydration procedures. The following changes occurred in the ascorbic acid content of the carrots during dehydration.

ASCORBIC ACID CONTENT OF CARROTS

Raw (mg./100 gm.)		Diced (mg./100 gm.)		Blanched (mg./100 gm.)		Dehydrated (mg./100 gm.)		Loss during dehydration procedures (pct.)
Moist	Dry	Moist	Dry	Moist	Dry	Moist	Dry	
7.5	68	5.7	50	3.8	28	21	22	32

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Cornell University.

[2] These analyses were made by K. W. Wheeler at the State Experiment Station, Geneva, New York.

The dehydrated carrots did not contain sufficient ascorbic acid for study. They were shipped to the laboratory packed in sealed, five-gallon tins in an atmosphere of carbon dioxide, and the cans were stored at —17.8°C.(0°F.) until cooking studies were run. Studies were completed within 12 weeks.

Sample bricks of the above dehydrated carrots were made, with a pressure of from 500 to 5,000 pounds per square inch, at the New York State Agricultural Experiment Station. After the optimum pressure was determined, through rehydration studies, to be 1,600 pounds per square inch, sufficient bricks were made at this pressure to run a cooking study consisting of five replications. The average weight of the bricks was 388 grams. The dimensions of the bricks were 4⅛ by 3⅛ by 1¾ inches.

EXPERIMENTAL PROCEDURE

Cooking: The dehydrated carrots were cooked in amounts to serve 50 men with two-thirds cup of cooked carrots per serving. About two pounds of carrots or exactly 964 gm. were used in the first three studies. About one and three-fourths pounds or exactly 908 gm. (the amount given in Swift's Army Cook Book) of carrots were used in the fourth and fifth cooking studies. Each method was repeated five times.

Study 1: The carrots, in 964-gm. lots, were added to 7,200 gm. of water and heated to boiling temperature in a stock pot on an institution-type gas range. From one to two minutes were required to return the water to boiling. The carrots were then simmered slowly for 30 minutes.

Study 2: The carrots in 964-gm. lots were added to 7,200 gm. of water at 20°C.(68°F.) in an aluminum dishpan. This was placed in an institution-type steamer preheated to 98.9°C.(210°F.). After two and one-half minutes, the pressure was brought up until it registered five pounds and the carrots were steamed at this pressure for 15 minutes. The pressure was reduced to zero pounds in one minute and the carrots were then removed from the steamer.

Study 3: An army field range was used. The carrots in 964-gm. lots were added to 7,200 gm. of rapidly boiling water in a heavy aluminum stock pot. The water required two minutes to return to boil. The carrots were boiled for 25 minutes. It was impossible to maintain a simmering temperature with this range.

Study 4: The carrots in 908-gm. lots were soaked in 5,450 gm. of cold tap water for 20 minutes. The carrots and soaking water were then placed in a stock pot on an institution-type gas range and heated until the water started to boil. They were then simmered for 20 minutes.

Study 5: The carrot bricks used in this study were soaked in 5,450 gm. of cold tap water for 90 minutes. The carrots and soaking water were put into an aluminum stock pot and heated on an institution-type gas range. About 45 minutes were required for the water to reach boiling. The carrots were simmered for 20 minutes.

Holding: A portion of the carrots and cooking water from Method 4 was held in an aluminum stock pot on top of the gas range for one and one-half hours with only sufficient heat to keep them warm [about 65°C.

(149°F.)]; another portion of carrots and cooking water was held under the same conditions for three hours.

Sampling: Analyses of the dehydrated carrots were made before each cooking study; losses because of cooking were calculated from these results in order to rule out the effect of any changes in content because of storage or of variation from can to can.

The dehydrated carrots were ground in a Wiley mill to 60 mesh before sampling. Samples for vitamin analysis were taken in duplicate. Aliquots of refreshing and cooking waters, as well as vegetable samples, were analyzed for their vitamin content.

Methods of analyses were as follows:

Carotene was determined by a modification of the Moore and Ely (1941) method by Ellis, Bernstein, and Thompson (1943). This method consisted essentially of four steps: (1) extraction of the vegetables by a petroleum ether-ethanol mixture; (2) transference of the extracted pigments to petroleum ether; (3) chromatographic separation of the extracted pigments using a column containing a mixture of starch and Super-Cel; (4) dilution of the carotene and colorimetric determination using Corning filters 038 and 554. Two-gram samples of the dehydrated carrots and 10-gm. samples of the rehydrated carrots were used. Because preliminary analysis of the cooking water showed no measurable amount of carotene, the cooking water was not analyzed.

Thiamin and riboflavin determinations were made by the Conner and Straub (1941) method as modified by the thiochrome method as adapted for vegetables by Moyer and Tressler (1942). Five-gram samples of the dehydrated carrots, 25 gm. of the rehydrated carrots, and 50 ml. of the cooking water were made to volumes of 500, 500, and 100 ml., respectively, before 50-ml. aliquots were passed through the adsorption apparatus.

Niacin determinations were made by the microbiological method of Snell and Wright (1941). Water extractions were made of two-gram samples of dehydrated carrots, 10 gm. of rehydrated carrots, and 10 ml. of the cooking water. The extracts were made to a volume of two liters. Three- and five-ml. aliquots were used.

Total solids were determined by drying five gm. of ground dehydrated carrots for six hours, and 30 gm. of rehydrated carrots for 48 hours, at 70°C.(158°F.) under 28 to 30 inches of vacuum.

Palatability Tests: Carrots prepared by the five methods described above were scored for flavor, texture, and appearance by at least six experienced judges. Five points were allowed for each factor so that the maximum score was 15.

Statistical Treatment: In order to determine whether the observed differences in the effects of the different cooking and holding methods on the vitamin content of the various vegetables were real or merely due to experimental variation in the method of study, a statistical analysis of representative data was made.

The per cent retention in the vegetable as served, calculated on the moist basis by using the weight of the carrots at each stage of the preparation, was selected for the statistical treatment as the figure of greatest importance in the studies. Standard errors were calculated and used in

ascertaining by the "t-value" method as outlined by Snedecor (1938) the significance at the five-per cent level of the difference in the observed retention of each vitamin between (a) the different cooking methods, (b) the different holding methods, and (c) the cooking methods and the holding methods.

DISCUSSION OF RESULTS

The effect of five methods of preparation on the carotene, thiamin, riboflavin, and niacin content of dehydrated carrots is given (Table 1). The

TABLE 1

Effect of Five Methods of Preparation on Carotene, Thiamin, Riboflavin, and Niacin Content of Dehydrated Carrots[1]

Method of preparation	Total solids	Carotene	Thiamin	Riboflavin	Niacin
	pct.	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
1. Dehydrated carrots	94	79.5	.42	.40	2.1
Brought to simmer in stock-pot (1-2 min.), simmered 30 min	15	112.3	.34	.41	1.4
2. Dehydrated carrots	96	71.6	.41	.47	2.4
Steamed 15 min. in steamer	18	92.7	.30	.41	1.3
3. Dehydrated carrots	95	76.5	.64	.77	2.6
Boiled 25 min. in Army field range	19	86.7	.39	.54	1.7
4. Dehydrated carrots	95	78.3	.39	.47	2.5
Swift's Army Cook Book, soaked 20 min	28	79.6	.39	.49	2.2
Brought to boil (45 min.), simmered 20 min	18	73.6	.34	.48	1.7
Held 1½ hr. over low gas flame (60-70°C.)	18	62.5	.34	.55	2.0
Held 3 hr. over low gas flame (60-70°C.)	17	81.4	.36	.58	2.0
5. Dehydrated carrot bricks	98	75.5	.40	.57	3.3
Soaked 90 min	21	102.4	.39	.47	2.5
Heated to boil on gas range (45 min.), simmered 20 min	17	105.6	.35	.51	3.0

[1] Vitamin content is reported on the dry basis. Each study was repeated five times.

dehydrated carrots contained an average of 76.3 mg. of carotene, 0.45 mg. of thiamin, 0.54 mg. of riboflavin, and 2.6 mg. of niacin per 100 gm. on the dry basis.

The effect of the five methods of preparation on the retention of carotene, thiamin, riboflavin, and niacin and the statistical treatment of these data are given (Table 2).

No appreciable loss of carotene occurred during the first three methods of cooking. Some loss occurred in Methods 4 and 5 in which the vegetables were soaked before cooking. This loss may have been caused by slight diffusion into the cooking water as was apparently true also in a study of rutabagas by Fenton, Barnes, Moyer, Wheeler, and Tressler (1943). This assumption is indicated by the increased retention of carotene when the

TABLE 2

Effect of Five Methods of Preparation on Retention of Carotene, Thiamin, Riboflavin, and Niacin in Dehydrated Carrots[1]

Method of preparation	Average vitamin retention ± S.E.[2] and ranges							
	Carotene		Thiamin		Riboflavin		Niacin	
	Carrots	Liquid	Carrots	Liquid	Carrots	Liquid	Carrots	Liquid
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
1. Dehydrated carrots brought to simmer in stock pot (1-2 min.), simmered 30 min.	102±2.64 96-108	.....	57±.43 55-60	33 30-38	72±4.96 55-85	39 31-46	48±2.24 43-55	60 58-63
2. Dehydrated carrots steamed 15 min. in water	95±1.04 91-97	.....	52±.98 49-57	30 21-39	64.5±1.4 63-67	31 22-38	42±5.89 21-57	37 25-47
3. Dehydrated carrots boiled 25 min. in Army field range	99±3.88 93-114	.....	52±2.93 47-62	11 7-15	61±7.22 48-89	13 7-20	58±2.47 51-63	20 11-26
4. Dehydrated carrots								
(a) Swift's Army Cook Book, soaked 20 min.	88 68-111	.....	76 70-77	26 23-35	75 65-85	21 20-23	58 51-63	25 23-29
(b) Brought to boil (45 min.) simmered 20 min.	81±5.96 66-102	.....	75±1.71 70-77	26 21-30	89±2.69 80-95	29 27-36	57±2.95 50-64	28 21-37
(c) Held 1½ hr. over low gas flame (60-70°C.)	80±6.46 65-98	.....	88±3.16 80-96	13 12-18	118±12.61 92-170	13 12-18	80±6.80 59-100	12 11-18
(d) Held 3 hr. over low gas flame (60-70°C.)	108±11.27 90-140	.....	88±4.06 73-95	12 9-18	118±3.98 104-125	12 7-15	80±4.07 66-89	10 5-14
5. Dehydrated carrot bricks (a) bricks soaked 90 min.	102 95-108	.....	73 63-77	31 29-33	61 53-64	22 19-27	57 53-61	36 35-37
(b) Heated to boil on gas range (45 min. required), simmered 20 min.	89±1.10 86-91	.....	57±1.73 51-61	48 44-52	55±11.91 48-66	39 31-44	58±5.79 46.80	54 48-58

[1] Per cent retention and solution were calculated on the moist basis using weights of uncooked dehydrated vegetable, cooked vegetable, and cooking water. Each study was repeated five times. [2] Significant differences in retention were found between:

Carotene	Thiamin	Riboflavin	Niacin
4b and 1,2,3	1 and 2	1 and 2,3,4b,5b,4c,4d	2 and 1,3,4b,4c
5b and 1,2	4b and 1,2,3,5b	4b and 3,5b	4c and 1,3,4b
1 and 4c	4c and 1,2,3,4b,4d	4c and 2,3	4d and 1,2,3,4b
	4d and 1,2,3,4b	4d and 2,3,4b	

cooked vegetable was held three hours. Upon evaporation of the cooking water some of the diffused carotene may have been adsorbed by the vegetable.

The thiamin retention in the carrots in all cooking methods was about the same (from 52 to 57 per cent) except in Method 4 (75 per cent). A smaller proportion of cooking water was used in Methods 4 and 5. No appreciable destruction of thiamin occurred in any of the cooking methods, with the exception of Method 3. In this method the water boiled because it was impossible to maintain a simmering temperature.

The average retention of riboflavin in the carrots ranged from about 55 to 89 per cent. As with thiamin, the highest retention occurred in Method 4. No appreciable destruction of riboflavin occurred in any of the cooking methods with the exception of Method 3. As in the case of thiamin, no explanation is given.

TABLE 3

Vitamin Content and Palatability Score of a Serving (100 gm.) of Dehydrated Carrots After Five Methods of Preparation

Preparation	Vitamin content				Palatability score [1]
	Carotene	Thiamin	Riboflavin	Niacin	
	mg.	*mg.*	*mg.*	mg.	
1. Carrots added to boiling water, returned to boil (1-2 min.), and simmered 30 min.....	17.3	.053	.063	.22	11
2. Carrots added to cold water and steamed 15 min. in steamer.....	16.4	.053	.073	.24	11—
3. Carrots boiled 25 min. in Army field range..	16.5	.074	.104	.33	13
4. Carrots soaked 20 min., brought to boil (45 min.), and simmered 20 min. according to Swift's Army Cook Book directions..	13.0	.060	.086	.29	11+
Held 1½ hours over low gas flame (60-70°C.).....	11.9	.062	.100	.36	....
Held 3 hours over low gas flame (60-70°C.).....	13.8	.062	.098	.35	....
5. Carrot bricks soaked 90 min., heated to boil on gas range (45 min.), simmered 20 min.	18.1	.060	.088	.51	11

[1] The possible score was 15, 5 each for appearance, texture, and flavor.

The per cent niacin retention did not differ greatly among the methods used, although Method 2 resulted in the least retention. Over one-half of this vitamin was retained; little destruction occurred.

The carrot bricks contained more niacin but about the same amount of carotene, thiamin, and riboflavin as did the loose carrots. The bricks were soaked and cooked soon after they were pressed, hence no studies were made on what is presumably their greatest advantage besides small volume, namely retention during storage of those vitamins subject to oxidation. Although the bricks were cooked by a method similar to the one used for the loose carrots (Method 4) as advocated in the Swift's Army Cook Book, they retained lower percentages of thiamin and ribo-

flavin than did the loose carrots. This might have been because of the longer soaking period required for the bricks to break apart or because the pieces were somewhat crushed by pressing.

Holding the carrots at 65°C. in their cooking water resulted in an increase in the per cent retention of each of the four vitamins in the carrots themselves, and a decrease in the cooking water. This was probably because of a change in the vegetable-liquor ratio. No actual destruction of any of the vitamins occurred during holding. On the contrary, an apparent increase in the riboflavin was noted.

The vitamin content and palatability score of a serving (100 gm.) of the carrots after the five methods of preparation are given (Table 3). A hundred-gram serving of the prepared carrots contained from 13 to 18.1 mg. of carotene, from 0.053 to 0.074 mg. of thiamin, from 0.063 to 0.10 mg. of riboflavin, and from 0.22 to 0.51 mg. of niacin.

To test the accuracy of the chemical method used, samples of the carrots and cooking water were analyzed in another laboratory by the microbiological method of Snell and Strong (1939). The percentage differences obtained by the two methods were within experimental error (Table 4).

TABLE 4

Comparison of Snell and Strong Method With Conner and Straub Method for Determining Riboflavin Content of Carrots

Description of vegetable	Riboflavin content [1]		Percentage differences
	Snell and Strong method [2]	Conner and Straub method [3]	
	mg./100 gm.	*mg./100 gm.*	
Cooked dehydrated carrots	.091	.095	4.2
Cooking water	.073	.066	9.6
Cooked and held dehydrated carrots	.088	.094	6.4
Cooking water from held carrots	.076	.073	4.0

[1] All data reported on moist-weight basis. [2] Snell and Strong analyses made by Dr. F. W. Tanner, Jr., at the State Experiment Station in Geneva, New York. [3] Conner and Straub analyses made in this laboratory as part of a regular cooking study.

Palatability: The palatability ratings for the carrots cooked by different methods were very similar, 11 to 13 points out of a possible 15 (Table 3).

SUMMARY

The carotene, thiamin, riboflavin, and niacin contents of dehydrated carrots, both in the loose and in the brick form, were determined (Table 1).

The percentage retentions of the vitamins in four methods of cooking the loose carrots and one method of cooking the carrot bricks are reported (Table 2).

Retention of the vitamins during one and one-half and three hours of holding (65°C.) are also reported.

Little, if any, of these four vitamins was destroyed. Most of the carotene, except in Methods 4 and 5, was retained in the vegetable; from 52 to 75 per cent of the thiamin, from 55 to 89 per cent of the riboflavin, and from 42 to 58 per cent of the niacin were retained. Most of the rest of the three water-soluble vitamins was found in the cooking water.

Method 4 resulted in slightly better retention of the water-soluble vitamins, thiamin and riboflavin, perhaps because of the smaller amount of cooking water used and the longer cooking period required. With longer cooking greater evaporation of the water occurred.

Holding the carrots warm for serving apparently resulted in some adsorption of each of the vitamins from the cooking water.

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RETENTION OF ASCORBIC ACID IN STRAWBERRIES DURING PROCESSING, FROZEN STORAGE, AND MANUFACTURE OF VELVA FRUIT

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Satisfactory retention of ascorbic acid during freezing and storage of strawberries has been reported by Fellers (1936), Kirk and Tressler (1941), and Bogdanova (1940). Fitzgerald and Fellers (1938), however, found averages of only 21 and 13 mg. per 100 grams in samples from two commercial plants. Rabak (1939) found that strawberries vacuum-packed in enameled cans contained 25 mg. per 100 grams after seven years of storage at —9°C.(15°F.) but only 13 mg. per 100 grams when sealed at atmospheric pressure and held at the same temperature. Practically no ascorbic acid remained in samples packed in paraffined paper cups with or without added sugar.

Mayfield and Richardson (1943) have recently reported large losses. Their sliced, sugared berries lost 10 per cent during freezing and storage for less than two weeks and 46 to 57 per cent during freezing and storage for two months. After four months only 18 to 23 mg. per 100 grams remained; the loss was 53 to 72 per cent. They also found that whole berries in 65-per cent sirup lost up to 22 per cent during the freezing alone, and that the loss reached 34 to 45 per cent during two months and 65 to 69 per cent during six months of frozen storage. In general, Mayfield and Richardson found the loss most rapid during the first two months. From the end of the second week to the end of the second month, the concentration of ascorbic acid increased in the sirup but decreased sharply in the berries. After two months the concentration was higher in the sirup than in the berries, although the reverse had been true just after freezing. Mayfield and Richardson used tightly sealed glass jars, typical of home rather than commercial freezing.

Dernovskaya-Zelentsova and Dylevskayo (1941) have pointed out that slow defrosting during 24 hours causes a loss of 40 to 50 per cent of the ascorbic acid of unsugared strawberries. In sugared berries the loss was variable (5 to 45 per cent).

Antonov and Svereva (1940) found 58 to 71 mg. per 100 grams in fresh strawberries. During freezing and six to eight months of storage the unsugared berries retained 49 to 61 per cent of the ascorbic acid originally present, those in 50 per cent sugar sirup retained 67 per cent, and those with dry sugar retained 75 to 84 per cent. Of the original ascorbic acid, 14.4 per cent was lost during thawing.

Reports thus do not wholly agree on the effect of modern commercial practice on the ascorbic acid of frozen strawberries. This paper reports

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studies on losses of ascorbic acid from frozen strawberries owing to condition of fruit and during freezing, short storage, extended storage, defrosting, puréeing, and manufacture of Velva Fruit.

PROCESSING METHODS

Three groups of samples were used in the experiments reported here. The first group, used in studies on the effect of condition of fruit before freezing and effects of extended frozen storage and defrosting, was prepared in a commercial plant in Gresham, Oregon. In this area strawberries are picked and hulled during the early part of the day and frozen during the afternoon and evening. The Marshall is the only variety. They are washed in mechanical washers and the adhering moisture and extraneous matter are shaken off on vibrating slat shakers. Samples were taken from the end of the sorting belt and packaged in one- and two-pound commercial containers — that is, heat-sealed, moisture-vapor-proof cellophane liners and waxed cardboard boxes. The berries were frozen (1) whole with added sugar sirup in a 3+1 ratio, (2) whole without sugar, and (3) sliced with added dry sugar usually in a 4+1 ratio. The packages were frozen in a freezing tunnel. The unsugared berries were frozen on trays and packaged after freezing. The samples were shipped to the Western Regional Research Laboratory by commercial refrigerated truck.

The second group of samples, which were used in the experiments on losses during freezing and short storage, was prepared at the Western Regional Research Laboratory. A numbered experimental variety from a late-season November harvest was used. The berries were hulled after they had been washed and sorted. Heat-sealed, moisture-vapor-proof cellophane bags and waxed cardboard boxes of half-pound size were used. Only 200 grams were placed in each package. A portion of the whole berries was frozen in the field boxes, without washing or hulling, and packaged later. These samples were frozen and stored in moving air at —23°C.(—10°F.).

The third group of samples was of the Banner variety, grown in the Santa Clara valley of California. This group was used in the experiments on puréeing and manufacture of Velva Fruit. They were puréed in a pulper equipped with a conical screw expeller and screen, which prevents excess incorporation of air. A portion of the purée was flash-pasteurized in equipment consisting of a positive-action rotary pump; a steam-jacketed, stainless-steel, tubular heater; and a water-jacketed, stainless-steel, tubular cooler. A temperature of 82 to 85°C. (180 to 185°F.) was reached after 15 seconds of exposure to heat. The purée was cooled in the same time interval to approximately 49°C.(120°F.) and frozen in moving air at —23°C.(—10°F.) in gallon glass containers. Individual samples were also packaged in moisture-vapor-proof liners and waxed boxes for testing purposes.

ANALYTICAL METHODS

The ascorbic acid determinations were made according to the method of Loeffler and Ponting (1942). The packages were first defrosted at room temperature; the time required was about four hours. The drained weights were determined on the whole berries after two minutes of drain-

ing on 8-mesh screens. Duplicate samples of at least 50-gram size were taken of both the sirup and the drained berries and immediately extracted in either one per cent metaphosphoric or one-fourth per cent oxalic acid. Ponting (1943) has shown that oxalic acid is suitable for ascorbic acid determinations in frozen strawberries. The sirup and the drained berries were thus tested separately. The amount of ascorbic acid per 100 grams of packaged material was calculated from the amounts found in the juice and in the drained berries and from the respective amounts of juice and berries—that is, from the drained-weight determinations. The figures given (Tables 5, 6, and 7) are averages of determinations on individual packages; those in the other tables are averages of the determinations on more than one, usually three or four, individual packages. The titratable acidity was determined by an electrometric titration to pH 8.0 with a glass-electrode pH meter. It has been calculated as citric acid.

EFFECT OF CONDITION OF FRUIT BEFORE FREEZING

Berries may arrive at the processing plant in varying degrees of maturity and may be delayed between picking and freezing. Delay was simulated by permitting berries to stand 48 hours on the covered loading platform of the freezing plant at Gresham, Oregon. They were then handled according to good plant practice—that is, they were washed and carefully sorted, and soft, moldy, or rotten berries were discarded. The "delayed" samples were obtained from the remaining good berries.

TABLE 1

Effect of Condition of Fresh Fruit on Ascorbic Acid Content of Whole Strawberries in 50-Per Cent Sirup (3+1 Ratio) After Three Months of Storage at −23°C.(−10°F.)

Condition	Drained weight	pH		Citric acid		Ascorbic acid		
		Liquid	Berries	Liquid	Berries	Liquid	Berries	In 100 gm. of pack
	pct.			*pct.*	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg.*
Grade A	55.7	3.29	3.21	.69	.79	42.1	55.1	49.4(44-54)[1]
Fresh wet	54.1	3.45	3.42	.49	.69	34.0	53.1	44.2(39-50)[1]
After 48 hr. delay[2]	50.7	3.47	3.45	.43	.52	36.1	47.4	41.2(40-42)[1]
Immature[2]	62.2	3.28	3.18	.51	.99	35.7	70.4	58.8(57-60)[1]
Overmature[2]	56.0	3.45	3.60	.47	.59	41.1	53.6	48.0(45-51)[1]

[1] Figures in parentheses represent maximum range of individual ascorbic acid determinations.
[2] The delayed samples were part of the "fresh wet" fruit. The immature and overmature samples were sorted from the same fruit as the "Grade A."

The samples designated (Table 1) as "fresh wet" were fresh, mature berries harvested after a rainy period. The berries had higher water content under such circumstances. The "Grade A" packs were prepared later in the season during drier weather. The berries delayed before freezing were from the same stock as the "fresh wet" packs, while the immature and overmature packs were sorted out from the same fruit as the "Grade A" packs. Maturity was judged by color.

Commercially packed Marshall strawberries in 50 per cent sugar sirup contained on an average more than 45 mg. of ascorbic acid per 100 grams, or 66 mg. per 100 grams on a sirup-free basis, after three months of frozen

storage (Table 1). Since the highest value found for fresh, mature strawberries in this laboratory has been 80 mg. per 100 grams, it is likely that the loss during freezing and three months of storage was less than 14 mg. per 100 grams, or 17.5 per cent.

The packs of immature berries were especially high in ascorbic acid, averaging in the whole berries about 70 mg. per 100 grams, or over 90 mg. per 100 grams on a sirup-free basis. The mature and the overmature packs had less but were about equal to each other. Delay, accompanied by good sorting, did not reduce the content greatly but delay should be avoided to reduce waste and prevent softening of the berries, loss of juice, and mold.

The degree of maturity is indicated by the pH and the citric acid determinations. The acid remaining in the drained whole berries was almost twice as much in the immature as in the overmature berries. The degree of maturity of the sliced berries (Table 2) was also estimated by an objective color determination on clarified filtrates with a photoelectric colorimeter. The units are a function of the intensity of pigmentation or the degree of absorption of light of a complementary wave length. The immature berries had an index of nine units; the fresh-wet, Grade A, and delayed berries had indices between 13 and 15; the overmature berries had an index of 24 to 28 units.

TABLE 2

Effect of Condition of Fresh Fruit on Ascorbic Acid Content of Sliced, Sugared Strawberries After Three Months of Storage at —23°C.(—10°F.)

Condition	pH of blend	Citric acid in blend	Soluble solids in blend	Ascorbic acid	
				In blend [1]	Corrected for added sugar
		pct.	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*
Grade A (3+1)	3.37	.58	30.5	43.5(43-45)	58
Fresh wet (4+1)	3.39	.57	23.4	45.7(44-48)	57
After 48 hr. delay (4+1)	3.43	.53	25.7	44.3(43-46)	55
Immature (4+1)[2]	3.20	.90	23.9	61.9(61-63)	77
Overmature (4+1)[2]	3.39	.63	25.3	54.4(53-57)	68

[1] Data in parentheses show maximum range of results. [2] These packs were prepared at a different time; hence the berries were not comparable with the other packs represented in this table.

The trend of the data on sliced berries (Table 2) is essentially the same as that shown by the analyses of the whole berries in sirup. Packages of sliced berries, frozen and stored three to four months under commercial conditions, contained more than 40 mg. of ascorbic acid per 100 grams, or more than 50 mg. per 100 grams on a sugar-free basis. Delay before freezing, accompanied by sorting to remove rotten and moldy berries, did not reduce the ascorbic acid greatly. The immature berries were especially high in ascorbic acid, averaging more than 75 mg. per 100 grams on a sugar-free basis. There was little decrease during the change from mature to overmature; the latter, in fact, averaged almost 70 mg. per 100 grams with correction for the added sugar.

EFFECT OF FREEZING AND TWO MONTHS OF STORAGE

Of each 1,100-gram portion of fresh berries of the second group of samples, 100 grams were tested for ascorbic acid before freezing, 400

TABLE 3

Effect of Freezing and Two Months' Frozen Storage at −23°C.(−10°F.) on Ascorbic Acid of Whole Strawberries

Before freezing	Ascorbic acid (mg. per 100 gm.)						
	After freezing			After storage			Approx. total loss (pct.)
	In blend [1]	Corrected for added sugar	Approx. loss (pct.) [2]	In blend [1]	Corrected for added sugar	Approx. loss (pct.) [2]	
			Dry (no sirup)				
63.0	63 (62-64)	63	0	54 (53-56)	54	14	14
			Water (no sirup)				
55.1 [3]	34 (30-37)	45	19	....	....	....	....
			Sirup, 40-per cent concentration (3+1)				
55.1 [3]	35 (31-38)	47	15	....	....	....	....
			Sirup, 50-per cent concentration (3+1)				
55.1 [3]	37 (36-38)	50	10	35 (34-38)	47	5	15
			Sirup, 65-per cent concentration (3+1)				
55.1 [3]	38 (38-38)	50	9	37 (34-40)	49	2	11

[1] Data in parentheses show range of results. [2] These are values obtained after correction for added sugar. Thus the loss per 100 gm. of packaged mixture was even less. [3] The values for the 400 gm. of fresh whole berries have been averaged. The individual results ranged from 53.1 to 56.4 mg. per 100 gm.

TABLE 4

Effect of Freezing and Two Months' Storage at −23°C.(−10°F.) on Ascorbic Acid Content of Sliced Strawberries

Before freezing	Ascorbic acid (mg. per 100 gm.)						
	After freezing			After storage (−10°F.)			Approx. total loss (pct.) [1]
	In blend	Corrected for added sugar	Approx. loss (pct.) [1]	In blend	Corrected for added sugar	Approx. loss (pct.) [1]	
			No added sugar, soluble solids 8.5 per cent				
Av. 53.1	54 (51-56) [2]	54	0	48 (48-48) [2]	48	11	11
			Added sugar (8+1), soluble solids 18.3 per cent				
Av. 53.1	41 (41-42) [2]	47	12	40 (39-41) [2]	45	4	15
			Added sugar (4+1), soluble solids 25.7 per cent				
Av. 53.1	39 (38-40) [2]	48	9	38 (38-39) [2]	48	1	10
			Added sugar (1+1), soluble solids 49.7 per cent				
Av. 53.1	24 (23-25) [2]	48	10	24 (23-26) [2]	49	0	10

[1] These are values obtained after correction for added sugar. Thus the loss per 100 gm. of packaged mixture was even less. [2] Maximum range of individual results.

grams (two packages) were tested within one week after freezing, and the remaining 600 grams (three packages) after two months (Tables 3 and 4). Sampling error was reduced by blending the entire contents of the package. This was accomplished by splitting each 200-gram package into approximately equal halves (after about a half hour at room temperature) and adding each half to 650 ml. of oxalic acid extractant in preparation for defrosting, extracting, blending, and filtering. Portions of the filtrates from the two halves were then mixed before testing to account for any uneven splitting of the package.

No loss of ascorbic acid occurred during the freezing of the dry berries; the loss in berries frozen in 50- and in 65-per cent sirup was approximately 10 per cent. The loss was greater with the weaker sirup and water, but samples of the latter kind were more variable. During the first two months of storage the dry berries lost approximately 14 per cent of their ascorbic acid, while the sirup packs did not lose significant amounts. Sliced berries (Table 4) without added sugar lost no ascorbic acid during freezing but lost 11 per cent during the first two months of storage. Those with added sugar lost about 10 per cent during freezing but did not lose significant amounts during the first two months of storage. The loss was somewhat greater for those packed with less sugar, as compared with those packed with larger amounts, but the difference was not significant.

EFFECT OF EXTENDED STORAGE

The Oregon samples prepared under commercial conditions and tested after three months of storage (Tables 1 and 2) were tested again after 15 months of storage at—23°C.(—10°F.) (Table 5). Though the variation

TABLE 5

Ascorbic Acid in Whole and Sliced Frozen Strawberries After 15 Months of Storage[1]

Condition	Ascorbic acid	Average
Whole berries		
	mg./100 gm.	*mg./100 gm.*
Fresh wet	42.6, 43.5, 43.2, 45.8, 46.4	44.3
Delayed 48 hours	38.3, 34.5	36.4
Immature	54.1, 53.9	54.0
Overmature	49.8, 49.7	49.7
Sliced berries		
Sugar added (4+1)	46.3, 46.0, 46.4, 47.2, 48.0	46.8
Sugar added (3+1)	42.1, 44.1, 45.5	43.2

[1] Samples shown in Tables 1 and 2.

between samples does not permit exact calculation, the losses in the sirup packs were usually less than five mg. per 100 grams, or 12 per cent of the ascorbic acid present after three months of storage. The average loss, considering all samples of whole berries packed in sirup, was less than two mg., or five per cent. The sliced, sugared berries did not lose a detectable amount of ascorbic acid in the same time. The averages differed by less than one mg., or two per cent.

Types of Commercial Packs: Frozen samples from a uniform lot of the Oregon berries were compared as a means of estimating the relative retention of ascorbic acid in dry pack (without sugar), sliced berries with dry sugar, and sirup pack. As shown (Table 6) the dry pack and the sliced berries had slightly more ascorbic acid per 100 grams than the other pack. Corrections for the added sugar or sirup show that retention in the sugared and the siruped packs was much better than in the dry pack.

TABLE 6

Ascorbic Acid in Different Types of Commercial Packs of Strawberries After 15 Months of Frozen Storage

Soluble solids	Amount found	Corrected for added sugar
pct.	Dry (whole, no sugar)	
	mg./100 gm.	*mg./100 gm.*
7.2	46.9	46.9
7.3	47.3	47.3
	Av. 47.0	47.0
	Sliced, sugar added (4+1)	
23.2	47.2	58.9
21.2	48.3	60.3
.....	46.3	57.9
.....	46.0	57.5
.....	46.4	57.9
	Av. 47.0	59.0
	Whole, 50-per cent sirup added (3+1)	
.....	45.8	60.9
.....	46.4	61.7
.....	42.6	56.6
.....	43.5	57.9
.....	43.2	57.5
	Av. 44.0	59.0

Sugar Concentration: Because of the shortage of sugar it has been necessary to freeze strawberries with the least sugar that would preserve the quality, flavor, and vitamin content. Retention was shown to be equally as good with the smaller as with the larger quantities of sugar (Tables 3, 4, and 5). Additional packs were prepared at Gresham, Oregon, to show the effect of varying sugar concentration (Tables 7 and 8).

The retention of ascorbic acid in whole berries packed in sirup (Table 7) appears to be independent of density of sucrose, within wide limits. It was essentially the same in 20-, 40-, and 50-per cent sirup but slightly less in 65-per cent sirup. The flavor of the 50-per cent sugar-sirup pack was judged superior to that of the 65-per cent pack. The latter was so sweet that the desirable fresh-fruit flavor was masked. In no way did the 65-per cent sirup appear superior to more dilute sirup. The drained weight was no larger, the flavor no better, and leaching of ascorbic acid from the berries into the juice actually greater. In the sliced berries (Table 8) the losses were less than 12 per cent and appeared to be independent of the

TABLE 7

Effect of Sirup Density on Ascorbic Acid of Whole Strawberries Stored 18 Months at −23°C.(−10°F.)

Soluble solids (pct.)		Drained weight (pct.)	Ascorbic acid (mg./100 gm.)			Average in blend, corrected for added sirup	Retention in drained berries (pct.)
Juice	Berries		Juice	Berries	Blend		
			20-per cent sirup (3+1)				
11.7	10.7	50.8	34.1	49.9	42.1	55.3	59.4
12.0	10.7	49.8	33.8	48.2	41.0	.....	58.6
			40-per cent sirup (3+1)				
19.0	11.7	50.7	31.6	48.6	40.2	53.0	60.6
19.8	12.2	54.0	31.9	45.7	39.4	.....	58.9
			50-per cent sirup (3+1)				
25.0	13.2	54.9	35.5	49.7	43.3	54.6	58.4
25.4	11-12	50.0	32.6	44.7	38.7	.....	57.8
			65-per cent sirup (3+1)				
29.7	14.3	50.7	34.8	42.8	38.8	49.8	55.0
34.2	14.6	50.2	32.4	39.8	36.1	.....	55.1

sugar concentration to a great extent. The unsugared pack lost the most ascorbic acid.

Taste testers preferred a soluble-solids concentration in the sugar-packed, sliced berries of about 18 to 20 per cent, in comparison with stronger or weaker concentrations of sugar. This concentration can be obtained with about an 8+1 mixture, and from the standpoint of ascorbic acid retention there appears to be little objection (Table 8; cf. Table 4) to the use of this concentration rather than the 3+1 and 4+1 now widely used.

TABLE 8

Effect of Added Sugar on Retention of Ascorbic Acid in Sliced Frozen Strawberries After Three and After 15 Months of Storage

After 3 months		After 15 months			
Soluble solids (pct.)	Ascorbic acid (mg./100 gm.)	Soluble solids (pct.)	Ascorbic acid (mg./100 gm.)	Corrected for added sugar	Loss (pct.)
		No sugar added			
5.2	56(56-56)[1]	7.2	50(46-56)[1]	50	10
		Sugar added (10+1)			
16.3	58(56-60)	16.9	56(55-58)	62	3
		Sugar added (8+1)			
16.0	55(54-56)	17.8	53(52-53)	59	4
		Sugar added (5+1)			
25.2	51	25.9	45(41-49)	53	12
		Sugar added (4+1)[2]			
.....	48	.....	47	59	2
		Sugar added (3+1)			
31.3	44(43-45)	28.0	43(42-46)	58	1
		Sugar added (2+1)			
.....	44(44-44)	35.9	39(38-40)	58	10

[1] Data in parentheses show range of determinations. [2] Samples were prepared from different lot of fruit from rest of table.

EFFECT OF SLOW DEFROSTING

Losses would be expected from strawberries held after being defrosted or during defrosting. From tests with a few samples (Table 9) it is evident that about a fifth of the ascorbic acid was lost from sugared, sliced berries but almost half from unsugared berries during long defrosting. Sugar has a similar protective effect during the defrosting of purées (Table 12).

TABLE 9

Effect of Defrosting on Ascorbic Acid Content of Frozen Strawberries

Prefreezing treatment	Defrosting at room temperature		Very slow defrosting		Approx. difference
	Ascorbic acid	Corrected for sugar	Ascorbic acid	Corrected for sugar	
	mg./ 100 gm.		*mg./ 100 gm.*		*pct.*
Whole, no sugar added	54	54	31[1]	31	43
Sliced, sugar added (8+1)	40	45	33[1]	38	17
Sliced, no sugar	50	50	28[2]	28	44
Sliced, sugar added (4+1)	47	59	36[2]	44	24

[1] Defrosted 24 hours at 4.4 to 10°C.(40 to 50°F.) and five hours at 26.7°C.(80°F.), after two months' frozen storage. [2] Defrosted 72 hours at 1.7°C.(35°F.), after 15 months' frozen storage.

PURÉEING AND MANUFACTURING OF VELVA FRUIT

Ascorbic acid was retained very well during puréeing and flash-pasteurization (Table 10), even though the purée was not deaerated before it was heated. The purée was tested immediately after the pulping, and changes during freezing and frozen storage are shown (Table 11).

TABLE 10

Retention of Ascorbic Acid During Puréeing and Flash Pasteurization[1]

Treatment	Ascorbic acid	Average
	mg./100 gm.	*mg./100 gm.*
Whole strawberries	76.6, 77.2, 78.8	78
Strawberry purée	76.0, 78.5	77
Flash-pasteurized purée	74.9, 71.3	73

[1] Tested immediately after berries were treated.

A portion of the purée was frozen with added sugar in a 3+1 ratio and part without sugar. The samples were frozen in the small cellophane and cardboard containers. The losses during freezing and storage were moderate; however, the samples were not strictly comparable with commercial bulk packages, since the latter are usually 30 pounds in size.

The loss of ascorbic acid during the freezing and storage of the flash-pasteurized purée includes that lost during the pasteurization. The pasteurized purée lost only 6.5 mg., or about nine per cent, during the freezing and storage alone. This loss indicates, however, that pasteurization at the temperatures used, 82 to 85° C. (180 to 185° F.), decreased but did not prevent the destruction of the ascorbic acid.

TABLE 11

Retention of Ascorbic Acid in Frozen Strawberry Purée After Three to Seven Months of Storage at —23°C.(—10°F.)

After 3 months' storage				After 7 months' storage				
Soluble solids (pct.)	Ascorbic acid (mg./ 100 gm.)	Corrected for added sugar	Loss [1] (pct.)	Soluble solids (pct.)	Ascorbic acid (mg./ 100 gm.)	Corrected for added sugar	Loss 3rd to 7th mo. (pct.)	Total loss [1] (pct.)
Unsugared								
9.2	65(65-65)[2]	65	16	10.3	57(55-59)[2]	57	14	27
Sugar added (3+1)								
32.7	51(51-51)	68	12	33.4	46(45-46)	61	10	21
Flash-pasteurized								
10.0	67(66-67)	67	14	.....	60	60	10	22

[1] Based on the fresh, unfrozen value of 77.3 mg. per 100 grams. [2] Data in parentheses show range of determinations.

Slow Defrosting of Purée: It seemed desirable to note the retention of ascorbic acid during defrosting of purée in a cool room at a slow rate, as is customary in commercial practice. Samples of the purée (Table 11) were tested after they had been held for 24 and 96 hours in small packages at 1.7°C.(35°F.). A portion was removed after the 24-hour defrosting and held in open containers in a household refrigerator at 4.4°C.(40°F.). Part of this portion was analyzed 24 hours later and another part 48 hours later. The results (Table 12) show that sugared purée (3+1) did not lose appreciable ascorbic acid during extended defrosting at cool temperature for 48 to 72 hours, but unsugared purée lost a considerable amount. In either case no significant loss occurred during 24 hours of defrosting at 35°F.

Manufacture of Velva Fruit: Frozen strawberry purée is suitable for use in the manufacture of a new frozen-fruit dessert, called Velva Fruit,

TABLE 12

Loss of Ascorbic Acid During Slow Defrosting of Purée

Defrosting time		Ascorbic acid		Loss during defrosting
35°F.	40°F. (open)[1]	Average	Corrected for added sugar	
Unsugared				
hr.	*hr.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*
0	0	56.7	56.7	0
24	0	54.1	54.1	5
24	24	46.9	46.9	17
24	48	39.1	39.1	31
96	0	34.7	34.7	39
Sugar added (3+1)				
0	0	45.7	60.8	....
24	0	45.3	60.4	1
24	24	43.2	57.5	5
24	48	41.8	55.8	8.5
96	0	33.6	44.8	26

[1] Samples held 24 hours at 1.7°C.(35°F.) in packages were held this additional time at 4.4 to 10°C.(40 to 50°F.) in open containers.

that has been developed at the Western Regional Research Laboratory, Loeffler (1944). It consists of fruit purée, enough sugar to make a soluble-solids content of 33 to 38 per cent, and gelatin. This mix is frozen in an ice cream freezer, and an "overrun" of 100 per cent, approximately the air content of ice cream, is introduced.

For these experiments strawberry purée frozen and stored in gallon glass jars was used. The jars of purée were defrosted by being held under running tap water and then the required additional sugar and gelatin were mixed in with a household-type mixer. The mix was then frozen in a small-batch ice cream freezer and packaged and hardened in 3½-ounce waxed-paper cups. Samples were analyzed within a few days without being defrosted (Table 13).

TABLE 13

Loss of Ascorbic Acid During Manufacture of Velva Fruit

Purée before manufacture[1]		After manufacture		
Ascorbic acid (mg./100 gm.)	Corrected for added sugar	Ascorbic acid (mg./100 gm.)	Corrected for sugar and gelatin	Loss during manufacture[2] (pct.)
No sugar in purée before manufacture				
67	65	37	58	12
Sugar in purée (3+1)				
51	68	44	65	4
46	61	39	58	4
Flash-pasteurized purée				
67	67	38	57	14

[1] These values are from Table 11. [2] These losses are the differences between the amounts in the Velva Fruit and in the small packages of the purée represented in Table 11. The losses indicated may have occurred partly during the relatively long defrosting of the jars; hence the loss during the manufacture of Velva Fruit may have been less than is indicated here.

The total losses of ascorbic acid during manufacture of Velva Fruit from the usual commercial pack with sugar added in a 3+1 ratio were very moderate (approximately 16 per cent). These total losses included the loss during puréeing, freezing, storage for three months, slow defrosting overnight at 35°F., mixing at room temperature, and refreezing with a high "overrun." The loss during defrosting of a gallon jar of the 3+1 mix and manufacture into Velva Fruit was less than five per cent. In other words, 84 per cent of the ascorbic acid originally present in the fruit was available to the consumer. Velva Fruit prepared from bulk purée stored for six months had 39 mg. per 100 grams when freshly prepared. After the Velva Fruit had been stored three weeks, it contained over 38 mg. per 100 grams. No significant loss occurred in these samples or in the other berry Velva Fruit samples stored several months. These quantities of ascorbic acid thus represent the amount actually available to the consumer.

This excellent retention of ascorbic acid during freezing storage of Velva Fruit indicates that it is an excellent vehicle for fortification with ascorbic acid. Samples of Velva Fruit made with other berries, fortified to hold 75 mg. of ascorbic acid per 100 grams, have not lost ascorbic acid during storage for six months at —10°F.

DISCUSSION

A loss of 10 to 15 per cent of ascorbic acid occurred during the freezing of strawberries, both whole in sirup and sliced with added sugar. This loss during freezing was greater than the loss during pulping or during the application of considerable heat in flash-pasteurization. The loss during freezing was less when no sugar was added but greater and certainly more variable when water rather than sugar was added.

Sugar undoubtedly tends to draw the juice, including ascorbic acid, from the berries by osmosis before the freezing. The greater loss can then be explained on the premise that the ascorbic acid is more susceptible to oxidation in the liquid than in the berries. The difference may not be due simply to increased surface, since no significant loss occurred in the sliced berries frozen without added sugar.

When water is added, the juice is not extracted to the same extent as it is by added sugar, but undoubtedly much of the ascorbic acid is carried into the liquid. It is possible that, if some of the ascorbic acid is in a liquid phase, the addition of sugar will reduce the loss prior to and during the freezing operation. In purées in which the berries were definitely disorganized, the loss during freezing and the first three months of storage was reduced by added sugar. The smaller loss during freezing in the dry pack may have been due in part to more rapid freezing than in the sirup or water pack.

The effect of sugar in reducing loss of ascorbic acid is most pronounced, however, during defrosting, particularly if defrosting is slow or if the fruit is held even at low temperatures after it has been defrosted. The loss during such treatment is reduced at least half by the presence of sugar. The losses during extended defrosting or holding surpass the losses during freezing and frozen storage.

After storage for three months (Table 1) or after 15 months, the juice contained a concentration of ascorbic acid that averaged only 65 to 75 per cent of that in the drained berries. With immature berries the concentration in the juice was less than 50 per cent of that remaining in the drained fruit. Apparently the firm texture of the immature fruit reduced leakage and the berries retained both a higher drained weight and a higher ascorbic acid content. An equal concentration of ascorbic acid in berries and sirup as reported by Mayfield and Richardson (1943) was encountered in these samples only on extended defrosting or holding at cool temperatures after defrosting.

It is likely that the losses of ascorbic acid during the freezing of the berries are not due to enzymic processes. If enzyme systems capable of destroying ascorbic acid were present, then large losses would have occurred during the pulping operation. Such losses (up to 100 per cent) have occurred in fruits containing enzyme systems that cause darkening and in melons containing ascorbic acid oxidase. It is likely therefore that the losses during freezing and defrosting are autoxidative in nature.

In contrast, whole berries in sirup and sliced berries in dry sugar retained ascorbic acid very well during both the two-month and the 15-month storage periods. The losses were hardly large enough to be significant, but the berries packed without added sugar were losing ascorbic

acid at a significant rate. Hence sugar or sugar sirup apparently reduces autoxidation in the frozen state. Since retention was almost equally good in dilute sugar concentrations, smaller ratios can be utilized than are now being used commercially.

The very high retention during storage indicates that autoxidation was slowed down and almost completely stopped at a temperature of —10°F. The packs had a high degree of contact with oxygen. They were not air-tight or in hermetically sealed containers. They were not cased, as commercial samples would be, but stacked individually on shelves in a cold room having continuous mechanical blowers maintaining a high degree of air circulation.

This same prevention of autoxidation at —10°F. has been shown even more decisively by Velva Fruit samples of strawberries and other berries, which have not lost a detectable amount of ascorbic acid in storage up to six months despite intimate contact with air and oxygen introduced as "overrun."

With frozen strawberries there is no need for airtight, hermetically sealed containers. Strawberries in sirup or sliced in sugar apparently do not lose a significant amount of ascorbic acid during frozen storage as do some fruit products—for example, frozen orange juice, Loeffler (1941). The evaporation in the sirup packs was not over two to three per cent. This indicates also that the moisture-vapor-proof cellophane liners and waxed cardboard boxes in commercial use are satisfactory for frozen strawberries.

The high retention of ascorbic acid shown in these samples has undoubtedly been influenced by the uniformly low temperatures employed. If temperatures had tended to vary and increase occasionally, the same degree of retention might not have occurred. Under such conditions different types of containers might be more suitable.

SUMMARY

Effect of Condition of Fruit: Prime mature strawberries averaged 66 mg. of ascorbic acid per 100 grams on a sirup-free basis three months after freezing. Immature fruit under the same conditions averaged 91 mg. per 100 grams, while overmature fruit averaged 55 mg. per 100 grams. After storage, the concentration of ascorbic acid in the sirup was 65 to 75 per cent of that in the drained whole berries and remained about the same despite prolonged storage. The sliced berries had on the average more than 57 mg. per 100 grams on a sugar-free basis after three months of frozen storage.

Effect of Freezing: Whole or sliced strawberries frozen without added sugar or sirup did not lose ascorbic acid during freezing. Whole berries in sirup and sliced berries with dry sugar lost about 10 to 15 per cent of their ascorbic acid. Whole berries packed in water lost about 20 per cent of their ascorbic acid. Puréeing or flash-pasteurization of purée prior to freezing did not reduce the ascorbic acid content over five per cent.

Freezing and Short Storage: Whole berries in sirup and sliced berries with dry sugar lost less than five per cent of their ascorbic acid during the first two months of frozen storage. The berries without added sugar or

sirup lost 12 to 15 per cent. Purée with added sugar lost 12 per cent during freezing, three months of frozen storage, and defrosting. Without sugar the loss was 16 per cent.

Extended Storage: Strawberries frozen with sirup or sliced with dry sugar lost on the average about five per cent more of their ascorbic acid when the storage period was extended from three to 15 months at —10°F. (—23°C.). The retention was as good in whole berries packed in 50-per cent sirup as in sliced berries packed in dry sugar, but was less in berries packed without added sugar or sirup. This retention was equally good despite wide variations in the ratio of added dry sugar to the sliced berries or in the density of sirup added to whole berries. During the period from the end of the third to the end of the seventh month of frozen storage the unsugared purée lost 14 per cent and the sugared purée 10 per cent of the original ascorbic acid. In general, therefore, the losses in sugared berries were about 20 per cent during freezing, storage, and normal defrosting. (These data indicate that the consumer of frozen strawberries should obtain about 80 per cent of the ascorbic acid of the original fruit.)

Effect of Defrosting: If defrosting is slow or if the strawberries are held several days at low temperatures after defrosting, the loss may be much greater than that during freezing preservation. The loss in sliced berries was twice as great in the absence of sugar as in its presence. In purée, the loss was not significant when defrosting was accomplished in 24 hours at 35°F. If the defrosting and holding took 96 hours, the loss in the sugared purée was 26 per cent and in the unsugared purée, 39 per cent.

Velva Fruit Manufacture: When sugared purée was manufactured into Velva Fruit the loss of ascorbic acid was less than five per cent. Unsugared purée lost 12 per cent of its ascorbic acid during the mixing and refreezing.

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ORGANOLEPTIC TESTS PERTAINING TO APPLES AND PEARS[1,2]

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DESIRABILITY VERSUS TEMPERATURE

The object of this article is to present results of certain organoleptic tests carried out on several varieties of apples held in cold storage at 0.6°C. (33°F.) and in a room at 22.2°C.(72°F.) and also on fruits at various times of the day. During March and April of 1944 apples that had been in a refrigerator since harvesting were cut into halves. One half of each apple was then kept at room temperature for about 24 hours while the other half was left in the refrigerator. Each half was then cut into five or six slices and served to judges who graded each sample by the following score sheet, which is called Score Sheet X.

Score Sheet X GRADING CHART FOR FRUIT

Sample No._____ Date_____

Factor	7	6	5	4	3	2	1
	Very desirable	Desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Undesirable	Very undesirable
A							
B							

Which sample is juicier?

Name_____

Sample A was a slice of the half which was held in a storage room at 33°F. while Sample B was a slice from half of the same apple kept at room temperature during the previous 24 hours. Each judge had a part of an apple at 72°F. and a part of the same fruit at 33°F. The fruits were served within 15 or 20 minutes after they were removed from their respective storage rooms. No appreciable change in temperature could have taken place during this short time. Records were kept of the scores on each fruit as well as the scores on Samples A and B. The numbers in the "factor" row of Score Sheet X were given the terms *very desirable, desirable,* etc.

Tests were made on two well-known varieties of apples, Delicious and Grimes. The judges were composed of young women in a co-operative house, a home economics class of young women, secretaries, and faculty men and women at Michigan State College; averages of the scores made by the entire group are shown (Table 1).

[1]A progress report.

[2]Journal article No. 752 (n.s.) Michigan Agricultural Experiment Station, East Lansing, Michigan.

The judges did not know how the samples had been treated nor did they know that Samples A and B were from the same apple. Many of the judges did not realize that one sample was colder than the other, a few did. Each judge appeared to be putting forth a serious effort to distinguish between the samples and to register his decision. Each judge realized that a comparison was to be made between Samples A and B.

Correlation coefficients between scores relating to A and B were calculated to determine whether or not there was a relation between what the judges thought about A and B. There was no association between scores on A and B for the Delicious apples. There was a definite relationship between the two sets of scores pertaining to the Grimes apples.

TABLE 1

Averages of Scores[1] for 103 Judges in Tests Made on Apples Held at 72 and 33° F.

Variety	33°F.	72°F.
Delicious	6.12±.10	4.84±.12
Grimes	5.37±.10	4.87±.11

[1] Grade points.

The averages (Table 1) pertaining to the samples at the lower temperature were significantly larger than those pertaining to the higher temperature. This was true for both varieties. There were no significant differences between the group averages; hence the scores for all groups were combined. These results were the decisions of 103 mature people.

Several of the judges, after being told the nature of the test, suggested that apples which had been kept in refrigeration from harvest to April would change quickly on being taken out of the refrigerator and apples that were taken out of the refrigerator and left for 24 hours at room temperature were not comparable to those left in refrigeration. This suggestion indicated that the former fruits had changed chemically during the 24 hours previous to testing and that the latter had not. With this suggestion in mind it was decided to carry out similar tests in the fall of 1944 as the fruits were harvested.

TABLE 2

Averages of Scores[1] in Desirability Tests Made on Apples Held at 72 and 33°F.

Variety	33°F.	72°F.	Number of judges
McIntosh	6.28	5.66	36
Fameuse	6.19	5.69	36
Grimes	5.62	4.69	26
Wealthy (small)	4.68	3.96	23
Wealthy (large)	5.85	5.15	26

[1] Grade points.

The tests were all made within the two-weeks period following harvest. The varieties used were Fameuse, McIntosh, Grimes, and Wealthy. In every one of the tests carried out on mature apples, those tasting them preferred the samples at the lower temperature. Averages of these tests

(Table 2) show that the averages of scores on the fruits at refrigeration temperature were significantly larger than the averages of scores pertaining to the fruits at room temperature.

JUICINESS VERSUS TEMPERATURE

Near the left-hand corner of Score Sheet X is a question concerning juiciness of Samples A and B. Percentages of the judges who said that the fruit at the lower temperature was juicier than that at the higher temperature, percentages of those who thought that the fruit at the lower temperature was not juicier, and percentages of those who had no preference are shown (Table 3); these records are for four panels of judges who tasted the samples in the spring of 1944. Most of the samples were sliced from the fruit about 15 minutes before the judges ate the samples. These samples were kept covered until the tasters were served. A large majority of samplers thought that the cold fruit was juicier than the fruit at room temperature. More than 85 per cent of all the judges considered Sample A juicier than Sample B, or thought that apples held at 33°F. were juicier than those at room temperature.

TABLE 3

Judges' Choice of Juiciness of Apples—Spring 1944

Preference	Panel of judges				Total weighted
	1	2	3	4	
	pct.	*pct.*	*pct.*	*pct.*	*pct.*
Apples at low temperature juicier	70.8	91.3	84.0	96.0	85.1
Apples at low temperature not juicier	16.7	8.7	12.0	4.0	10.3
No preference expressed	12.5	0.0	4.0	0.0	4.6

Percentages of the judges' ratings for juiciness and weighted total percentages for apples tested in the fall of 1944 (Table 4) show that 69 per cent thought that fruits held at 33°F. were juicier than those at room temperature; 19 per cent thought that those at room temperature were juicier, while 12 per cent had no choice. These percentages are lower than those obtained in the spring. The difference may be due to varieties and different stages of development. A possible explanation of the differences in weighted averages for Tables 3 and 4 may lie in the fact that

TABLE 4

Decisions Concerning Juiciness of Apples Held at 72°F. Versus Those at 33°F.—Fall 1944

Variety	Those who thought cold fruit was juicier	Those who thought fruit at room temp. was juicier	No preference
	pct.	*pct.*	*pct.*
Jonathan	68	18	14
McIntosh	69	25	6
Snow	75	19	6
Grimes	69	19	12
Wealthy	70	22	8
Total weighted	69	19	12

apples gradually lose moisture in most storages. A moisture loss of two to four per cent in five or six months is common. Both treatments sampled in the fall were actually juicier than those in March and April.

CONSISTENCY OF JUDGES

To determine whether or not the judges were consistent in making their decisions two groups of judges, totaling 52 people, were given Samples A and B, where A and B were from the same apple at room temperature. Score Sheet X was used for recording decisions. The average pertaining to Sample A was not significantly different from the average pertaining to Sample B. This was true for both groups. The two sets of averages are as follows:

GROUP 1 Jonathan		GROUP 2 McIntosh	
Sample A	4.60	Sample A	3.91
Sample B	4.61	Sample B	3.62

These values indicate that the judges were consistent. On examining the individual score it was seen that the differences between the scores for Samples A and B were small. The fact that the judges' decisions were about the same on these homogeneous materials strengthens the significance found on the other tests relating to fruits at room temperature and at 33°F.

EXAMINATION OF SCORE SHEETS

Appropriate score sheets should help judges make their decisions. All people interested in detecting differences in food qualities by organoleptic tests are not agreed on the best type of score sheet. Some desire an elaborate one on which several categories are listed together with many descriptive adjectives; they believe that a detailed score aids the sampler in recording his decision. Others believe that this type of score sheet forces the judge to record his opinion where he does not want to record it.

The following score sheet, Score Sheet Y, was used in some of the later tests to determine whether or not a judge could make a decision employing a card with two classifications as well as one with seven.

The left-hand end of the line joining *Very poor* and *Excellent* in this grading chart was considered to have a value of zero; the right end was

GRADING CHART FOR FRUIT

Score Sheet Y

Sample No._____ Date_____

Place a short vertical line across the horizontal line joining Very poor and Excellent where in your opinion the sample should be classified.

A_____

Very poor Excellent

B_____

Very poor Excellent

Which sample is the juicier?

Name_____

considered to have a value of 6, for the line was six inches long. Points between *Very poor* and *Excellent* were given values equal to the distance from the zero point. By using this method of recording judgments, scores did not come out in whole numbers as they did for Score Sheet X. After a decision was made with regard to Sample A or B it was rather easy to decide where the other sample should be recorded. The judge was left with the responsibility of recording his decision about the second sample after placing a vertical line to represent his score for the first sample. This responsibility involved placing a vertical line for the second sample to the left or to the right of his first score and determining how far to the left or to the right of the former this line should be placed. This type of grading sheet required each taster to think about the two samples in relation to each other and forced him to attempt a decision concerning the second sample in contrast with his conclusion about the first.

Percentages of responses of the tasters concerning these two score sheets (Table 5) indicate that the tasters liked Score Sheet X better than Score Sheet Y. A chi-square test applied to the totals indicates that these percentages are significantly different from the percentages 33 1/3, 33 1/3, 33 1/3 which would occur if there were no preferences concerning these score sheets. It indicates also that a judge prefers descriptive classifications in rendering a decision concerning desirability of an apple. The terms *Very desirable, Desirable,* etc. in Score Sheet X make it easier for the sampler to evaluate apple quality of one fruit in relation to another.

TABLE 5

Judges Favoring Score Sheets Y and X

Panel	Y over X	X over Y	No preference
	pct.	*pct.*	*pct.*
1	29.2	50.0	20.8
2	28.0	68.0	4.0
Weighted percentages	28.6	59.0	12.4

The t-tests (not given) show that Sample A is preferred to Sample B for all groups using the two score sheets. An interesting thing about these two methods of evaluation of desirability of apples is that the t-values, pertaining to Score Sheet Y, are nearly twice as large as the t-values pertaining to Score Sheet X. This signified that the judges make a greater contrast of Samples A and B by using Y than by using X. In most of the grades the differences between A and B are greater on Y than on X. This suggests that, although the judges do not like Y as well as X, the person carrying out the tests gets more significant results from Score Sheet Y than from Score Sheet X. These tests were executed to determine the contrast, if any, between fruits at refrigeration temperature and fruits at room temperature.

VARIETY VERSUS TEMPERATURE

Since the above tests show that people consider cold apples more desirable than apples at room temperature, the question arose as to whether or not people would consider cold apples of inferior quality more desirable than apples of superior quality at room temperature. The American

Pomological Society rates Rhode Island Greening, Baldwin, and Steele Red apples on a scale of 1 to 10 for dessert quality at 7-8, 5-6, and 8-9, respectively. For this study Rhode Island Greening and Baldwin apples at cold-storage temperature were compared with Steele Red at room temperature. On the first test Rhode Island Greening was Sample A while Steele Red was Sample B. On the second test, Baldwin was Sample A while Steele Red was Sample B.

On examining the average scores for two groups of faculty men and women (Table 6) it is clear that the judges placed cold Rhode Island Greening apples in the same class with Steele Red at room temperature. The averages pertaining to these varieties are not significantly different, although the averages for both panels concerning the former variety are larger than for the latter.

TABLE 6

Average Scores[1] on Desirability of Rhode Island Greening and Baldwin Apples Held at 33°F. and Steele Red Apples at Room Temperature

Variety	Panel 1	Panel 2
Rhode Island Greening	5.38	5.80
Steele Red	5.12	5.68
Baldwin	5.77	6.16
Steele Red	5.04	4.92

[1] Grade points.

The results of tasting cold Baldwin and Steele Red fruits at room temperature showed that the two panels of judges preferred cold Baldwin to Steele Red apples at room temperature. In both cases the means were significantly different. These tests indicate that low-rating fruits might be substituted for high-rating fruits by serving them cold.

SELECTING JUDGES

It is well known that individuals react differently to taste, smell, and other senses and that some individuals are not able to taste or smell unless a flavor is very pronounced or an aroma strong. Instances have been cited where judges were not consistent. On examining each score in the above tests there was very little evidence of poor tasters and only two or three cases where inconsistency appeared. The standard deviations were relatively small, showing that the scores fell in rather small ranges.

BEST TIME OF DAY FOR MAKING ORGANOLEPTIC TESTS

Some research workers in food laboratories believe that the best time of the day for judging food qualities is between two and three o'clock in the afternoon. Some think that the best time is immediately before a meal, preferably before the evening meal. There are some who consider the middle of the forenoon to be an appropriate time for making these tests. Many organoleptic tests are made at the time of the noonday meal. The tests described in this short paper were designed to determine the best time of the day for judging taste.

BEFORE VERSUS AFTER EVENING MEAL

Apples that had been in a refrigerator since harvest were cut into halves; one half of each apple was kept at room temperature for 24 hours,

while the other half was left in the refrigerator. Young women in a dormitory were served slices of these halves just before and immediately after the evening meal. Each judge was given a slice from half an apple at room temperature and a slice from the other half of the same apple which had been kept in the refrigerator. Grimes and Delicious apples were used in these tests. Score Sheet X was used for recording the decisions of the judges (Table 7). These college women were served a good meal and should not have been hungry after eating it.

TABLE 7

Average Scores[1] on Apples Eaten Before and After Supper

Variety	Before the meal		After the meal	
	Room temperature	Refrigerated	Room temperature	Refrigerated
Grimes	4.6	5.1	5.1	5.3
Delicious	5.6	6.6	5.1	5.9

[1] Grade points.

No one in the group knew the object of the test and did not suspect that he was eating slices of the same apple. There were no significant differences between the averages of the scores made before the meal and the averages of those made after the meal; this was true for both varieties. These results indicate that the time just before the evening meal is as good as the time immediately after this meal for tasting these varieties of apples and suggests that hunger or lack of hunger did not affect the judges' scores. This may be true also for other types of foods.

2:00 P.M. VERSUS 5:00 P.M.

Similar apples were served to a home economics class of young women who had had some experience in judging food qualities, at two and at five o'clock in the afternoon. At two o'clock they should not have been hungry; at five o'clock they should have begun to be hungry. The same type of score sheet was used for recording decisions (Table 8). There are no significant differences between the averages of scores made at two and at five o'clock. The results show that the time of day did not affect the decisions of the judges.

TABLE 8

Average Taste Scores[1] on Apples Eaten at 2:00 and at 5:00 P.M.

Variety	2:00 P.M.		5:00 P.M.	
	Room temperature	Refrigerated	Room temperature	Refrigerated
Grimes	5.11	5.56	5.17	5.72
Delicious	5.11	6.39	5.12	6.47

[1] Grade points.

RESULTS OF ORGANOLEPTIC TESTS MADE EVERY HOUR

Forty-seven secretaries at Michigan State College, who are mature people, were given slices of Jonathan apples every hour of the day from eight o'clock in the morning until five o'clock in the afternoon. The apples

were at room temperature when served. They were selected for uniformity and were about as homogeneous as could be obtained. The slices were carried to the secretaries and served to them at their desks. They ate the slices and recorded their decisions on the following score sheet:

GRADING CHART FOR FRUIT

Sample No._____ Date_____

Please taste the sample and record your decision by placing a check mark in the proper place.

7	6	5	4	3	2	1
Very desirable	Desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Undesirable	Very undesirable

Name_____

These Jonathan apples of excellent quality were enjoyed by all of the judges throughout the day. Most of them thought that the test was a variety test and did not suspect that the hourly samples came from a homogeneous source. Sometimes the fruits were divided into four parts, sometimes into eight parts, and sometimes into 12 parts; this varied the samples a little and made it appear that the hourly samples were not from the same source.

Most of the judges felt that after chewing the samples for a moment their impressions were more favorable than when they were ready to swallow the well-chewed material. It seems as though the first response of the taste buds to apples is much more desirable than later responses. The second decision concerning this fruit was not as good as the first.

It would have been impossible to get 47 professors, 47 students, or 47 workmen to be at one food laboratory at each hour of the day for one day. The only group of people on the campus who would be in the same places for eight hours was the group of secretaries. They could not leave their work to go to one laboratory 10 times during the day; hence the samples were taken to their desks. All samples were served on the hour or within five minutes of the hour so that the judges were tasting at intervals of about one hour throughout the day. This was a difficult experiment to carry out, for everything had to be done at regular intervals and all samples had to be in 27 different places about the same time for 10 times during the day. The judges were very co-operative during the entire experiment. Only one judge got "fed up" on apples and quit at three o'clock in the afternoon; the remainder liked the fruit and did not want to stop at the conclusion of the experiment.

One objection to the manner in which this experiment was carried out could be that the judges were not devoting all their time to judging apples. It is true that they were working at their tasks during the day but on each hour they took sufficient time to eat the samples and record their decisions. No doubt they would not have given more time to judging had they been in a food laboratory and done nothing but judge the samples at each hour

of the day. They appeared to be conscientious in judging. It seems as though this experiment was carried out in the most satisfactory way to determine whether or not one time of the day is best for judging food samples. At 8:00 A.M. and 1:00 P.M. the people had just eaten a meal. At 12:00 noon and 5:00 P.M. they were going to a meal and were more or less hungry. At 10:00 and 11:00 A.M. and 3:00 and 4:00 P.M. they were tasting in the between-meal period. Ranges, averages, and standard deviations of scores for the ten hours of the day are recorded (Table 9).

TABLE 9

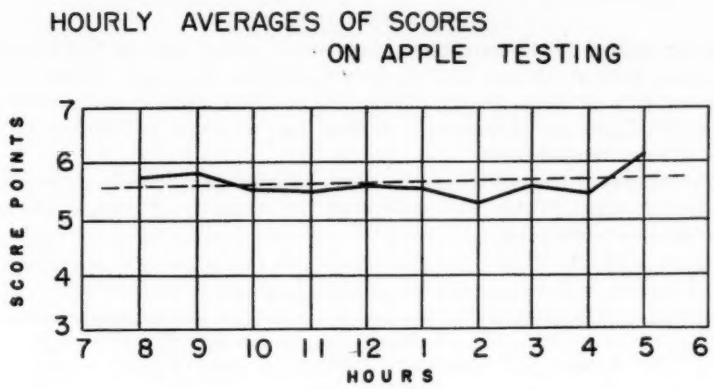
Hourly Ranges, Averages, and Standard Deviations of Scores on Jonathan Apples

	Hour					
	8:00 A.M.	9.00 A.M.	10:00 A.M.	11:00 A.M.	12:00 Noon	1:00 P.M.
Range	4	5	6	5	7	5
Av.	5.8	5.9	5.7	5.6	5.7	5.7
Std. dev.	0.91	0.94	1.15	1.10	1.20	1.26

	Hour				
	2:00 P.M.	3:00 P.M.	4:00 P.M.	5:00 P.M.	Av.
Range	6	5	5	5	5.3
Av.	5.4	5.7	5.6	6.2	5.7
Std. dev.	1.41	1.05	1.11	1.00	1.10

These averages and standard deviations are plotted (Fig. 1); an analysis of variance (not given) carried out on these data shows that there are no significant differences between the hourly averages. This means that as far as these people and Jonathan apples were concerned one hour of the day was about as good as any other hour for making organoleptic tests. The average score at 2:00 P.M. was smaller than those pertaining to the other hours; the average at 5:00 P.M. was larger than the others. There was no significant difference between the two o'clock average and the five o'clock average when tested with each other. The averages at 12 o'clock and at one o'clock were the same to one decimal place. There is no trend in the hourly averages over the 10 sampling times. The trend line is drawn in Fig. 1; it is nearly horizontal, showing that the averages did not increase or decrease appreciably during the day. The trend line for the hourly averages, omitting the data for five o'clock, is negative but not significant; it is nearly parallel to the horizontal axis.

Bartlett's homogeneity test was applied to the variances or standard deviations. This showed no evidence of heterogeneity of the variabilities of the hourly scores. This means that the amount of scatter of the individual decisions at the various hours was about the same throughout the 10 tasting times. The standard deviations of the results obtained at eight and nine o'clock are smaller than the others but are not significantly smaller. The judges' scores were between 4.4 and 7.0; most of the individual averages were between 5.0 and 6.0; most of the standard deviations were about 1.00.



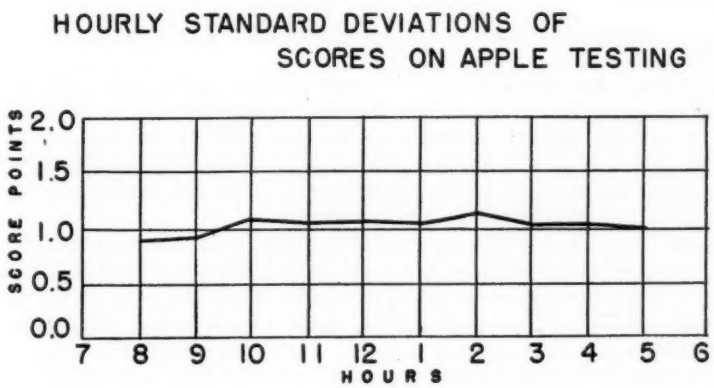


FIG. 1.

TABLE 10

Average Scores[1] of Tests on Pears at Room and at Refrigeration Temperatures

Variety	Held at 33°F.	Held at 72°F.
Bartlett	6.58	5.79
Bosc	5.69	5.19

[1] Grade points.

TESTS WITH PEARS

In the fall of 1944 organoleptic tests were carried out on Bartlett and Bosc pears held at 0.6 and 22.2°C.(33 and 72°F.). Averages of the scores on desirability of these fruits (Table 10) indicate that scores pertaining to the cold fruits are significantly larger than averages pertaining to the fruits at room temperature.

Percentages of the individual decisions concerning juiciness of these two varieties of pears (Table 11) show that the majority of judges thought the cold pears were juicier.

TABLE 11

Judges' Choice of Juiciness of Pears

Variety	Those who thought cold fruit was juicier	Those who thought fruit at room temperature was juicier	No, preference
	pct.	*pct.*	*pct.*
Bartlett	74.0	10.0	16.0
Bosc	65.4	15.4	19.2

[1] Grade points.

SUMMARY

This study has shown that the average mature person likes cold apples and pears better than those served at room temperature.

The average mature person also believes that fruits held at 33°F. (apples and pears) are juicier than similar fruits held at about 72°F.

A large majority of layman judges are consistent in their decisions concerning desirability of the fruits examined.

Score Sheet X is preferred to Score Sheet Y by the majority of the people used as judges, but the latter led to larger t-values than the former.

Cold fruits of inferior quality are considered by the average person to be about as good, if not better than, fruits of superior quality at room temperature.

One time of the day is about as good as any other time for tasting apples.

ASCORBIC ACID CONTENT OF THE MANGO IN RELATION TO VARIETY

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The mango is a fruit that can be grown in abundance in tropical and semitropical countries and with comparatively little difficulty of production. A review by the author (1943) of reported values shows that this fruit has a relatively high content of vitamin A, vitamin C, thiamin, and riboflavin. It serves as an important item in the diet.

In connection with a study of the ascorbic acid content of fruits of Puerto Rico carried out at the School of Tropical Medicine, Munsell (1945), the opportunity was offered of obtaining values for several varieties of mangoes with fruit samples carefully selected as to uniformity of quality and degree of ripeness.

EXPERIMENTAL PROCEDURE

Ascorbic acid was determined by the 2,6-dichloro-phenol-indophenol titration technique essentially as described by Bessey and King (1933). Extraction of the samples was made in a Waring blendor, as previously described by Munsell (1945), using 2.5 per cent metaphosphoric acid as the extractant. After blending, portions of the blend were centrifuged and aliquots of the clear liquid taken for titration. Dilution of the aliquots was made with eight per cent acetic acid as suggested by Musulin and King (1936).

The fruits were sampled for analysis by removing the skin over a narrow area from blossom end to stem end and cutting out a wedge-shaped slice. This was weighed and quickly transferred to the bowl of the blendor containing the measured amount of metaphosphoric acid solution. Blending was completed within one to two minutes. In selecting fruits for sampling care was taken to use only ripe fruits in prime condition. The analyses, in most instances, were made soon after the fruits reached the laboratory.

DESCRIPTIONS OF MANGO VARIETIES

With the exception of a few samples obtained in St. Thomas, Virgin Islands, all fruits were grown in Puerto Rico. The majority were produced under known conditions either at the Federal Agricultural Experiment Station at Mayagüez or at the Insular Agricultural Experiment Station in Rio Piedras. A few miscellaneous samples were purchased from itinerant vendors or were brought to the laboratory.

Detailed descriptions of the different varieties could be obtained only for those varieties sent from the Federal Agricultural Experiment Station. The following summary gives information relating to average size of

[1] The author wishes to express her appreciation to Miss Ana María Cuadros and to Miss Adelaida Elias for their assistance in making the analyses.

[2] Present address: Nutrition Laboratory, Pentagon Post Restaurants, The Pentagon, Washington, D. C.

fruit, flavor, amount of fiber, or other distinguishing feature for these 15 varieties.

Amini No. 2910—Average fruits weigh six to nine ounces. The color is yellow with heavy red or scarlet blush covering one-half to three-fourths of fruits growing on the outside of the tree. The flesh is a rich yellow with a strong aroma and relatively free from fiber.

Bulbulchasm No. 1835—Fruits are small, weighing about four ounces. The color is green to yellow. The flesh is yellow, juicy, and spicy but not fibrous.

Cambodiana No. 2106—A representative fruit weighs 10 ounces although there is considerable variation, some fruits weighing no more than five ounces while others weigh as much as one pound. The skin is smooth and of a rich yellow color. The flesh is pale or deep yellow, very juicy, and almost entirely free from fiber. This fruit is among the best in flavor and quality.

Climoise No. 3304—The skin is rose color and the flavor is like that of the mamey.

D. B. Alphonse No. 2215—Fruits average eight to 12 ounces in weight. The surface is smooth and yellow, shaded with pink on the side exposed to the sun. The flesh is yellow, firm, and juicy with a subacid flavor and has almost no fiber.

Fajri Long No. 2531—Fruits vary from medium to large, weighing on the average 14 to 15 ounces. The skin is smooth and green in color tinged with yellow and red. The flesh is deep yellow, sweet, and has good flavor and few fibers.

Haden No. 2914—Fruits vary from large to very large and weigh from 15 to 20 ounces and sometimes as much as 24 ounces. The skin is smooth and light to deep apricot-yellow in color, overspread with crimson-scarlet. The skin is very thick and tough. The flesh is yellowish-orange in color, firm, very juicy, fibrous only near the seed, and has a sweet, rich, moderately piquant flavor.

Martinique No. 1509—This fruit resembles the Sans Pareille closely. The fruit is inferior in flavor.

Paheri No. 3616—This fruit resembles the D. B. Alphonse but may be distinguished by the brighter red blush of the skin in fruits exposed to the sun. The flesh is slightly more tender and juicy and somewhat superior in flavor. Of the mangoes grown in Puerto Rico the Paheri ranks first in flavor.

Reine Amalie No. 3204—The fruit resembles the orange in color. The flesh is pale in color and has a rather insipid flavor.

Sans Pareille No. 2913—A good fruit weighs 14 to 16 ounces. The skin is smooth and of a dull, yellowish-bronze color. Much of the green color remains until the fruit is fully ripe. The skin is very thin. The flesh is rich orange at the apex and yellow at the base, tender, very juicy, and free of fiber. The flavor is rich and sweet, but strong and unpleasant.

Savoy No. 3206—This fruit resembles the Cambodiana. The skin is pale yellow in color. The flesh is yellow and fibrous and the flavor mild.

Singapuri No. 1215—The fruit is described as medium-large, weighing 10 to 11 ounces. The skin is uneven and light green to yellow in color

TABLE 1

Values for Ascorbic Acid Content of Two Fruits From Each of 28 Varieties of Mangoes

Source and variety of fruit	Ascorbic acid content (mg. per 100 gm. edible portion)	
	Fruit 1	Fruit 2
U. S. Department of Agriculture,[1] Federal Experiment Station, Mayaguëz, Puerto Rico		
Amini No. 2910	25.16	18.24
Bulbulchasm No. 1835	47.04	77.67
Cambodiana No. 2106	37.50	30.59
Climoise No. 3304	119.30	125.79
D. B. Alphonse No. 2215	70.41	21.55
Fajri Long No. 2531	15.74	7.15
Haden No. 2914	21.39	26.99
Martinique No. 1509	51.09	58.32
Paheri No. 3616	20.28	22.92
Reine Amalie No. 3204	65.90	75.19
Sans Pareille No. 2913	44.26	37.56
Savoy No. 3206	12.59	19.23
Singapuri No. 1215	7.35	6.87
Sufaida No. 2323	29.74	38.90
Totafari No. 2513	8.87	12.91
Insular Agricultural Experiment Station,[2] University of Puerto Rico, Rio Piedras, Puerto Rico		
Cambodiana	33.51	36.26
Cambodiana	26.85	23.85
Colombo Kidney	15.69	13.01
Colombo Kidney	15.66	16.94
Governor Ramos Seedling	94.06	65.89
Governor's Seedling No. 2	130.81	105.03
Mangotino Redondo	49.54	52.87
Manzano Sorce, Vieques Selection	17.62	16.84
Manzano Tete Nene	18.43	23.84
Mayagüezano	50.03	52.48
Charlotte Amalie, St. Thomas, Virgin Islands		
Seedlings resembling [3]		
(1) Mango D'Or	79.94	.....
(2) Mango de hilo	121.42	115.56
(3) Martinique	59.34	.....
Miscellaneous samples from Puerto Rico		
Mango pasote (common variety)	10.58	9.13
Mango piña	52.66	45.88
Variety not identified; grown near Ponce	16.26	17.72

[1] Supplied through the kindness of Mr. Milton Cobin. [2] Supplied through the kindness of Mr. Julio S. Simons. [3] Tentatively identified through the assistance of Mr. Arnold H. Winegar, Agricultural Experiment Station, U. S. Department of Agriculture, St. Thomas, Virgin Islands, and Mr. Julio S. Simons, Agricultural Experiment Station, University of Puerto Rico, Rio Piedras.

shading to orange at the base. The skin is thick. The flesh is light orange in color, fairly juicy, sweet and somewhat aromatic, and has little fiber.

Sufaida No. 2323—The fruit is large, ranging in weight from 24 to 36 ounces and sometimes to 40 ounces. The skin is smooth, very thin, and greenish-yellow in color. The flesh is dark yellow or orange near the apex, shading to lighter yellow near the seed and toward the base. The flesh is of good quality, of medium juiciness, and has a minimum of short, coarse fibers. The flavor is sweet, fairly rich, and pleasant.

Totafari No. 2513—An average fruit weighs 12 to 18 ounces. The skin is very smooth, light yellow in color, developing an uneven, bright pink blush when ripening exposed to the sun. The skin is thick and tough. The flesh is bright yellow, very juicy, and free of fiber, with a subacid, spicy, agreeable flavor.

RESULTS

Values were determined on two fruits for each of the 28 varieties studied. These are summarized (Table 1) according to the source from which the samples were obtained. Values for one unidentified variety are included.

DISCUSSION AND SUMMARY

Values for the ascorbic acid content of two fruits, each of 28 varieties of mangoes, are presented. The range of these values is from 9.13 milligrams per 100 grams of edible portion found for one fruit of the mango pasote or common variety to 130.81 milligrams per 100 grams, the value for one fruit of the variety designated as Governor's Seedling No. 2 and grown at the Insular Agricultural Experiment Station in Rio Piedras, Puerto Rico.

In the majority of cases the values for the two fruits of a given variety of mango are markedly similar. This similarity is in obvious contrast to the large differences between values for several of the varieties and even exists for samples from different localities. Fruits of the Cambodiana variety from Mayagüez and from Rio Piedras in Puerto Rico gave values within the range 30.59 to 37.50 milligrams, while those of the Martinique variety from Mayagüez and from St. Thomas, Virgin Islands, have values between 51.09 and 59.34 milligrams. The low values for the second lot of Cambodiana from Rio Piedras—somewhat lower than the range given above—is undoubtedly due to the fact that this lot of fruit was slightly overripe.

On the basis of the results presented, it seems justifiable to suggest that the vitamin C content of the mango is closely related to variety.

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FERMENTATION OF COFFEE

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Coffee is an extract of the roasted beans found in the berries or cherries of the coffee shrub *Coffea arabica*. The berries grow in clusters, each berry normally containing two seeds or beans surrounded by a parchment membrane (endocarp) and a soft pulp with an outside skin (exocarp). The separation of the beans from their coatings is accomplished in two ways. The whole berries may be dried in the sun and then run through a machine to remove the dried skin, pulp, and parchment membrane, or, in the wet method, the berries are passed through a pitting machine which removes the outer skin leaving the beans covered with the pectinous pulp and the parchment membrane. The berries without the skins are then placed in water and fermented to loosen the pulpy material, the pulp is washed away, and the beans are dried so that they may be hulled. In the latter process the dry beans are separated from the parchment membrane.

PREVIOUS STUDIES

Many types of seeds are separated from their immediate covering by fermentation. Tomato seeds, for example, were formerly separated from their pectinous coating by fermentation but later mechanical means were used. Loew (1907) studied the fermentation of cocoa beans and coffee beans. He reported the presence of bacteria and yeasts at the end of 16 hours and described the former as acetic acid bacteria. Although acetic acid was present, he found that adding this acid had little effect upon the slime. Picado (1926) observed differences in the quality of different coffees and thought that the fermentation process improved the quality of the coffee. Allen (1926) stated that the bacteria involved in the fermentation of coffee are similar to those found during the retting of flax. Thom and Church (1926) described several species of molds of the genus *Aspergillus* which were isolated from a coffee fermentation. They refer to the paper by Freudenberg (1920) in which bacterial activity is said to be inhibited by certain substances in coffee.

Lillienfeld-Toal (1931) isolated a mold, several species of yeast, and the following five species of bacteria from fermenting pulp: *Bacterium coli*, *Bacterium lactis aerogenes*, *Bacterium xylinum*, *Bacillus granulosis*, and *Bacillus vulgatus*. He observed that the bacteria of the coliform group grew best and thought that they were the cause of the acid production. However, he also observed that the slimy pulp was loosened before a high acidity was developed, and believed that the pulp may be loosened by the natural enzymes of the mature coffee.

MATERIAL STUDIED

Two samples of coffee berries were obtained in February, 1942, at Fortin, Mexico. At Las Animas hacienda, where the coffee beans were

being cleaned by the fermentation process, the berries were run through a pitting machine. The skins were discarded while the pits with the enveloping pulp were carried by a stream of water into a concrete vat. In this vat the pits were allowed to ferment for 18 to 20 hours. The pulp was then washed away from the pits and the pits were spread on a concrete platform to dry in the sun.

The samples were collected on a Monday morning. The first sample consisted of unbroken ripe berries collected in a sterile vial. The second sample consisted of the pits with the surrounding pulp collected in the same way from the trough which carried them to the vat in which they were fermented. The vials were sent on Tuesday morning from Mexico City, via air mail, and reached Geneva, New York, on Friday of the same week. These were cared for as described below.

The operator of the vat claimed that he was able to tell when the pulp would wash away from the pits by the acid smell that developed in the course of fermentation.

Additional samples of coffee cherries were secured in August of the same year through the courtesy of Sr. Carlos Madrid, Facultad de Agronomia, Medellin, Colombia, S. A., who had called at the Experiment Station in June. These cherries were sent on August 12th and arrived air mail on August 18th, unbroken and in good condition. They were handled as described below.

FERMENTATION OF COFFEE BERRIES FROM MEXICO

When the samples of unbroken berries were received from Mexico, they were brown to red in color and quite bright and apparently had changed very little. The other sample vial contained the pits and pulp. The latter was light green in color and apparently had changed but little. Both samples were partially covered with water and thus were protected from direct contact with air.

Parts of each of the samples from well down in the vial were immediately placed in sterile water, shaken thoroughly, and the water plated in several dilutions with a tryptone, yeast-extract, glucose agar. Plates were incubated two days at 32°C.(89.6°F.). Colonies were all small and white and grew mostly as subsurface colonies. A number of cultures were isolated from stabs made with the same agar. The colonies from the original red berries were given a "B" number and the second lot a "G" number.

Characteristics of the cultures were determined with observations on growth in stabs, on slants, in broth, gelatin, sucrose gelatin, and nitrate broth. Smears from broth cultures were stained by the Gram method and examined. Carbon dioxide production from glucose in tryptone, yeast-extract broth was determined in Eldredge tubes by absorption of the carbon dioxide with barium hydroxide. Fermentation characteristics on various carbon compounds were determined with the same basic medium. Selected cultures were grown in flasks of glucose broth with excess calcium carbonate using 300 c.c. in each flask. Later, these broths were analyzed

for alcohol and acetic and lactic acid, the type of lactic acid also being determined.

RESULTS AND DISCUSSION

All of the cultures grew well with a filiform growth in the stab but showed little growth on slants. Broth was clouded and after several days cleared with a light sediment. No growth was obtained in plain gelatin but in sucrose-tryptone, yeast-extract gelatin, beaded to filiform, growth was observed in all except one culture. Culture B14 produced the growth that is typical of the species *Leuconostoc mesenteroides* van Tieghem. All cultures except B14 were Gram-positive rods, of short to medium length, sometimes occurring in chains. All produced acid in glucose broth.

The cultures when grown in nutrient media containing the various carbon compounds were found to belong to three distinct groups. Eighteen of the cultures fermented nearly all test materials, produced acid, and curdled and reduced milk (Table 1, Group 1). Seventeen of the cultures averaged 0.54 per cent acid in broth with little carbon dioxide in Eldredge tubes (Table 2, Group 1). These cultures, B5, G13, and G2, produced inactive lactic acid as a primary acid product from glucose fermentation (Table 3). Such characteristics identify these cultures as strains of *Lactobacillus plantarum* (Orla-Jensen) Bergey *et al.* The group contains four cultures from the original red berries and 14 from the second or normal fermentation.

One culture, B14, was identified as *Leuconostoc mesenteroides* (Cienkowski) van Tieghem by its characteristic growth in sucrose gelatin, production of carbon dioxide (Table 2, Group 2), alcohol, acetic acid, and levo lactic acid from glucose (Table 3), and its acid production from carbon compounds (Table 1, Group 2).

Eighteen cultures produced acid from only the following carbon sources: xylose, arabinose, fructose, glucose, galactose, and maltose (Table 1, Group 3) and failed to acidify milk. They were found to be typically gas-producing lactobacilli in that they produced carbon dioxide (Table 2) and inactive lactic acid, acetic acid, and alcohol from glucose (Table 3, Culture B11). Since they fermented xylose and arabinose so readily but not sucrose or lactose, they were identified as strains of *Lactobacillus brevis* (Orla-Jensen) Bergey *et al.* Of the 18 cultures, 14 were isolated from platings of the original red berries and four from the fermenting berries.

The fermentation carried on in the samples was similar to that carried out in many fermenting vegetable materials. However, this does not necessarily indicate that this fermentation is essential to the breakdown of the pulp of coffee berries. It must be remembered that the samples were received several days after they were collected and had had opportunity to carry the fermentation further than is thought to be necessary under commercial conditions.

FERMENTATION OF COFFEE BERRIES FROM COLOMBIA

On August 18, 1942, a small lot of red (ripe) coffee cherries was received by air mail through the courtesy of Sr. Carlos Madrid, from Colombia, S. A. Although they were quite dry, some of these were immediately washed in sterile water, the water plated with a tryptone, yeast-

TABLE 1

Fermentation of Carbon Compounds by Organisms From Coffee Fermentation

Group No.	Number cultures	Acid produced	Glycerol	Xylose	Arabinose	Sorbitol	Mannitol	Fructose	Glucose	Mannose	Galactose	Sucrose	Maltose
1	18	Av.[1]	0.2	0.6	3.7	1.5	1.6	2.6	2.8	2.7	2.3	3.1	2.7
		Max.	0.2	2.8	5.5	2.1	1.9	3.4	3.7	4.0	2.9	4.7	3.7
		Min.	0.0	0.1	0.3	0.1	0.9	2.0	1.8	1.6	1.3	2.0	2.0
2	1	....	0.0	2.0	2.2	0.0	0.0	3.1	2.1	1.7	1.2	2.3	2.3
3	14	Av.	0.0	3.2	3.9	0.0	0.1	2.5	1.7	0.2	1.2	0.2	2.0
		Max.	0.0	5.2	7.7	0.0	0.5	3.4	2.4	0.6	1.5	0.4	2.7
		Min.	0.0	1.7	1.7	0.0	0.0	1.8	0.8	0.0	0.9	0.0	1.4
4	36	Av.	....	2.5	2.4	....	....	2.2	2.0	2.0	1.9	1.9	2.1
		Max.	....	3.0	3.4	....	....	2.8	2.3	2.2	2.1	2.3	2.4
		Min.	....	1.6	0.6	....	....	1.5	1.5	1.1	1.5	1.3	1.8
5	7	Av.	....	2.5	2.9	....	....	1.5	1.5	0.6	0.9	0.2	1.2
		Max.	....	5.0	5.2	....	....	3.0	2.6	1.8	2.0	0.8	2.5
		Min.	....	1.2	0.6	....	....	0.0	0.6	0.2	0.0	0.0	0.0
6	5	Av.	....	1.3	2.1	....	....	2.8	2.5	2.3	1.7	2.6	2.3
		Max.	....	1.4	2.9	....	....	5.4	4.6	4.4	2.0	3.2	3.8
		Min.	....	1.1	1.6	....	....	1.9	1.7	1.0	1.5	2.2	1.2

TABLE 1 *(Concluded)*

Group No.	Number cultures	Acid produced	Lactose	Raffinose	Inulin	Dextrose	Starch	Salicin	Amygdalin	Arbutin	Trehalose	Rhamnose	Milk
1	18	Av.	2.9	2.6	0	0.3	0.6	1.7	2.1	2.1	3.1	0.6	ACR[2]
		Max.	3.8	3.2	0	0.7	1.0	2.0	2.7	2.8	3.9	1.3	
		Min.	1.8	0.0	0	0.0	0.3	1.2	1.2	1.2	1.6	0.1	
2	1	....	1.6	1.4	0	0.4	0.5	0.8	0.6	1.5	1.7	0.0	A
3	14	Av.	0.1	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NC
		Max.	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
		Min.	1.2	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
4	36	Av.	2.0	0.0	....	....	....	....	....	....	....	....	ACR
		Max.	2.3	0.0	....	....	....	....	....	....	....	....	
		Min.	0.2	0.0	....	....	....	....	....	....	....	....	
5	7	Av.	0.1	0.0	....	....	....	....	....	....	....	....	NC
		Max.	0.8	0.0	....	....	....	....	....	....	....	....	
		Min.	0.0	0.0	....	....	....	....	....	....	....	....	
6	5	Av.	0.1	0.0	....	....	....	....	....	....	....	....	ACR
		Max.	0.7	0.0	....	....	....	....	....	....	....	....	
		Min.	0.0	0.0	....	....	....	....	....	....	....	....	

[1] Figures represent average (Av.) number of c.c. of N/9 acid produced in 10 c.c. of culture medium and maximum and minimum acid production in series (Max.) (Min.). [2] In milk recordings: A=acid, C=curdled, R=reduced, NC=no change.

extract, glucose medium, and the plates incubated two days at 32°C. A variety of colonies was present on the plates. About two per cent of the colonies were green mold, four per cent were of a tan-colored, aerobic, surface-colony type of bacterium, 10 per cent were of bacteria that produced white, aerobic surface colonies, and 84 per cent were of bacteria that formed pin-point, subsurface colonies. Forty colonies were isolated from the several plates. The isolates were studied to determine their nature (Table 4, Cultures 1 to 40).

Another lot of the coffee berries was placed in just enough sterile water to cover them and allowed to stand one day so that the skin could be removed readily. The brown-colored liquid had a medicinal odor. The coffee beans were separated from the skin with sterile forceps and the beans were placed in sterile water. This water was plated after one hour, two and a half hours, four hours, and 24 hours. Plate counts were 6,500,000, 11,400,000, 29,700,000, and 203,000,000 at these intervals. At the same time, the proportion of small, white, subsurface colonies increased from 54 to 74 to 90 to 100 per cent of the total colonies. Cultures were isolated as before (Table 4, Cultures 41 to 93).

Using the same methods as previously described, it was found that among the first 40 colonies examined, 15 were yeasts, one was a mold, five were contaminated, 12 were microaerophilic, Gram-positive rods and cocci, two were aerobic cocci, and five failed to grow (Table 4). During the fermentation the majority of the colonies isolated were found to be microaerophilic cocci and rods (Table 4). The yeasts, mold, and aerobic bacteria were discarded since it was the microaerophilic types that seemed to be predominant and to be of further interest.

However, the fermentation apparently was not exactly like the fermentation previously studied. Thirty-six cultures were almost identical in that they were Gram-positive cocci and fermented all of the sugars tested with about equal acid production (Table 1, Group 4) and acidified, curdled, and reduced milk. It is of interest that they fermented xylose and arabinose and produced more titratable acid from these sugars than from glucose. Twelve of the cultures averaged 0.216 per cent acid in glucose in Eldredge tubes and a comparatively small amount of carbon dioxide (Table 2, Group 4). Three selected cultures, C43, C46, and C58 (Table 3), produced dextro lactic acid as a major end product of glucose fermentation.

These characters seem to indicate that the cultures were of bacteria related to *Streptococcus faecalis* Andrewes and Horder. However, the high acid production from pentose sugars and failure to ferment raffinose were not observed by Andrewes and Horder (1906) nor by Orla-Jensen (1919).

Seven cultures in Group 5 (Tables 1 and 2) and C34 (Table 3) were found to be identical in every way with cultures in Group 3 and these were then identified as belonging to *Lactobacillus brevis* (Orla-Jensen) Bergey *et al.*

Five other cultures (Group 6, Tables 1 and 2) and Culture C22 (Table 3) were found to be identical to Group 1 and therefore were considered to be *Lactobacillus plantarum* (Orla-Jensen) Bergey *et al.* While they grew poorly in glucose broth in Eldredge tubes, the proportions of acid and carbon dioxide were similar to those in Group 1. This low acid production may have been due to the use of a poorly buffered medium.

TABLE 2

Average Carbon Dioxide Production From Glucose in Eldredge Tubes

Group No.	Acid and gas production in Eldredge tubes			
	Number cultures	Average acid	Average carbon dioxide	Carbon dioxide / acid
		pct.	*pct.*	
1	17	.540	.021	.04
2	1	.245	.091	.39
3	18	.335	.134	.40
4	12	.216	.019	.09
5	7	.136	.067	.49
6	5	.135	.007	.05

TABLE 3

End Products of Fermentation of Glucose by Representative Organisms Isolated From Coffee

Culture No.	End products from glucose			Zinc lactate		
	Alcohol	Acetic acid	Lactic acid	Water of crystallization	Specific rotation $(a)_D^{20°}$	Type of acid
	gm./ 100 ml.	*gm./ 100 ml.*	*gm./ 100 ml.*	*pct.*		
B5	.020	.146	2.228	18.15	....	Inactive
B11	.266	.107	0.668	18.25	....	Inactive
B14	.485	.078	1.016	13.15	7.3	Levo
G13	.020	.086	2.692	18.13	....	Inactive
G2	.012	.075	2.325	18.15	....	Inactive
C22	.025	.132	1.809	18.14	....	Inactive
C34	.309	.094	0.576	18.22	....	Inactive
C43	.035	.081	2.203	13.02	7.9	Dextro
C46	.018	.078	2.050	12.97	7.9	Dextro
C58	.020	.117	2.400	12.86	7.9	Dextro

TABLE 4

Grouping of Micro-organisms Isolated From Coffee Cherries and Beans From Colombia

Cultures	Isolated from	Number of isolates				Microaerophilic	Aerobic	Aerobic
		No growth	Yeasts	Mold	Contaminated yeast and bacteria	Gram+ cocci and rods	Gram + cocci	Rods
1-40	Original coffee cherries	5	15	1	5	12	2	....
41-51	Coffee beans—1 hour	1	....	....	....	5	2	2
52-63	Coffee beans—2½ hours	2	....	....	....	6	1	2
64-77	Coffee beans—4 hours	2	....	....	....	13	....	3
78-93	Coffee beans—24 hours	1	....	....	....	12	....	3

This fermentation was a lactic fermentation similar in nature to the lactic fermentation observed in the coffee cherries from Mexico, but the cocci present belonged to the genus *Streptococcus* rather than *Leuconostoc*. This difference is not surprising in that in the fermentation of other vegetable substances, streptococci have been found during the early part of the fermentation.

DISCUSSION AND SUMMARY

A bacteriological study has been made of the fermentation of coffee berries collected in Mexico and Colombia. Several types of bacteria, as well as molds and yeasts, were isolated, but as the fermentation proceeded, acid-producing bacteria became predominant. The fermentation is a typical lactic acid fermentation similar to that carried out in other vegetable materials and the species of bacteria isolated are the same as those isolated in other lactic acid fermentations, Pederson (1936, 1938), Hucker and Pederson (1930). Strains of *Leuconostoc mesenterioides* (Cienkowski) van Tieghem, *Lactobacillus plantarum* (Orla-Jensen) Bergey *et al.*, and *Lactobacillus brevis* (Orla-Jensen) Bergey *et al.*, and strains related to *Streptococcus faecalis* Andrewes and Horder were found. Although these organisms appear to be the types that produce the acid during the fermentation of the coffee berries, it does not necessarily mean that this fermentation is needed to bring about a breakdown of the pulp. However, in actual practice in the Las Animas hacienda, the operator claimed that he was able to tell when the pulp would wash away by the acid smell. The material studied was several days old when received, and the fermentation may have been carried further than it is carried in normal commercial practice.

It seems surprising to us that previous investigators should have laid so much emphasis on acetic acid fermentation, but perhaps this is natural as the volatile acids are recognizable by odor, while a laboratory determination is necessary in order to recognize the type of lactic acid present. All studies thus far made have been made with very limited facilities or materials.

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STUDIES ON VEAL. I. EFFECT OF DIFFERENT INTERNAL TEMPERATURES ON VEAL ROASTS FROM CALVES OF THREE DIFFERENT WEIGHTS

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In contrast to the many studies on proper methods of roasting beef, little work has been reported on similar projects for veal. Many housewives do not consider veal suitable for roasting, particularly veal from very young calves. Therefore, it seemed that a study of veal roasted to different internal temperatures might be valuable. Also, the physical characteristics of veal vary with the age and size of the animal, and the roasting directions for optimum results might vary accordingly. A project was set up to compare veal of three different sizes—small, medium, and large—roasted to four different internal temperatures—71, 77, 82, and 88°C.(160, 170, 180, and 190°F.).

The ideal veal roast, when cooked, should be reddish-brown on the outside and light gray inside. The meat should be juicy and tender, not dry and crumbly or wet and slippery. The recommendations given by various cookbooks for the proper internal temperature for well-done veal range from 65 to 85°C.(149 to 185°F.) with 77°C.(170°F.) most frequently given. Latzke (1934) and Latzke and Leeby (1930) gave 74°C.(165°F.), while Child and Bell (1930) used 71°C.(160°F.) in their work.

The general attitude on the cooking of veal is summarized in "Ten Lessons on Meat" (1943) as follows:

"In cooking veal, two inherent characteristics must be considered: (1) Lack of fat and (2) abundance of connective tissue. Veal is tender because it is the flesh of a young animal, but it contains considerable connective tissue and so requires long, slow cooking to make it tender."

Very few studies have been made on the actual amount of connective tissue in the muscles of animals at different ages. Mitchell, Hamilton, and Haines (1938) studied calves which had been on feed for various lengths of time and found no indication that age had any great effect upon the connective tissue content of the muscles analyzed. However, they stated that "much of the data obtained on this point were so irregular as to preclude any definite conclusions." Mackintosh, Hall, and Vail (1936) found that the collagen nitrogen was higher in mature than in yearling beef, and that the shear increased and the tenderness score decreased as the collagen nitrogen rose. Lowry, Hastings, Hull, and Brown (1942), in studying the rat, found that the collagen content of skeletal muscles decreased during the growth period.

EXPERIMENTAL PROCEDURE

Two pairs of veal legs from calves of each of three different weights were utilized in this study. The weights of the skinned, dressed carcasses

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approximated 50, 125, and 200 pounds, as representative of milk-fed calves (35 to 90 pounds), calves on first grass (90 to 150 pounds), and range-fed calves (150 to 275 pounds), respectively. All the animals were graded U. S. Good.

The carcasses were hung for five days at 1 to 2°C.(34 to 36°F.). The paired legs were then delivered to the kitchen and the muscles separated, wrapped, and stored at approximately 7°C.(45°F.) until cooked. The roasts were cooked on the three days following delivery, eight roasts being cooked each day.

The muscles used were the semitendinosus, semimembranosus, biceps femoris, gastrocnemius, vastus lateralis, gluteus medius, rectus femoris, adductor, and vastus medialis. The first three muscles were large enough to be divided into two roasts each, giving four roasts per animal. The

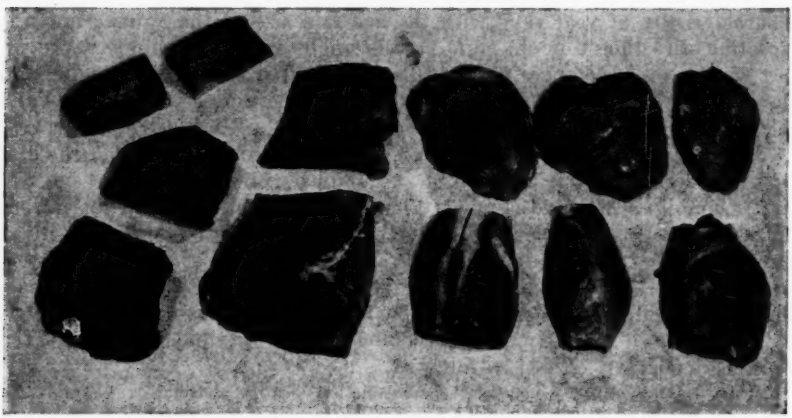

FIG. 1. Roasts prepared from muscles used in this study. 1, semitendinosus; 2, semimembranosus; 3, biceps femoris; 4, vastus lateralis; 5a, lateral head of gastrocnemius; 5b, medial head of gastrocnemius; 6, gluteus medius; 7, rectus femoris; 8, adductor; 9a, vastus medialis; 9b, vastus intermedius.

other muscles were grouped together as gastrocnemius-vastus lateralis, gluteus medius-rectus femoris, and adductor-vastus medialis; the roasts cut from these muscles are shown (Fig. 1).

The roasts were cooked in gas ovens at 163°C.(325°F.). The four internal temperatures used were assigned at random within each group of four cuts. The roasts were placed on racks in small open pans, and thermocouples were used to follow the changes in temperature during cooking. The roasts were cooked to the assigned internal temperature, checked for any rise in temperature after removal from the oven, then allowed to cool to room temperature before carving.

Sampling: Each roast was cut through the thermocouple hole, perpendicular to the direction of the fibers. One half was sliced for judging and for color comparison. The other half furnished samples for histological study and for shearing. The trimmings were used for digestion studies.

Judging: The roasts were judged for color, flavor, juiciness, texture, and tenderness by three to six judges. Each judge received one slice from each roast. The order of assigning the slices was kept constant so that the samples for each judge came from the same relative position each time.

The judging factors were scored on a rating scale of one to five, the highest score indicating the greatest desirability. In addition, judges also checked descriptive terms for color and texture.

Histological Studies:[2] Cooked muscles, 71°C.(160°F.), were sectioned on the freezing microtome at a thickness of 160 microns, and were stained with gentian violet to show the general distribution of connective tissue and to determine the arrangement of muscle fibers. Small pieces of raw muscle and of muscle cooked to 71 and 88°C.(160 and 190°F.) were fixed in formalin and Zenker's fixatives. The Zenker-fixed pieces were embedded, sectioned, and stained with Mallory's triple connective tissue stain, while the formalin-fixed pieces were embedded, sectioned, and stained with Weigert's elastic connective tissue stain.

Color: In an attempt to find an objective method of describing the color of the various roasts, samples were compared with the Munsell (1929) color plates.

Digetsion: No attempt was made to keep the muscles separate in the digestion work, as the samples available were not large enough to do so. The method used to determine comparative digestibility was adapted from Urbain, Wood, and Simmons (1942).

Statistical Analysis: The data were collected so that, in the statistical analysis, the effects of the temperatures, muscles, animals, and sizes could be separated. The temperatures and the sizes were the experimental variables. The variation between the muscles was of interest because the use of different muscles reduced materially the number of animals needed. It was hoped that there would be no difference between the two animals within any one size class, since they were intended as checks on each other.

RESULTS

Effect of Temperature: The average figures for cooking factors, judges' scores, and soluble nitrogen are given (Table 1) for the different internal temperatures studied.

Cooking Factors: The total cooking losses, losses by evaporation, and cooking time in minutes per pound increased steadily with increase in internal temperature. The drippings were chiefly water and water-soluble material as was reflected in the decrease in drippings above 77°C.(170°F.); above this temperature the water evaporated from the pan, leaving only the dried solids from the meat juices.

Judges' Scores: In general, the color, flavor, texture, and tenderness of the meat improved with each increase in internal temperature for all three sizes of animal. The extent of the improvement in color, texture, and tenderness decreased with increase in size, especially among the three upper temperatures. The juiciness decreased with increase in internal temperature.

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[2] We are indebted to Dr. E. J. Strandine, Research Laboratories, Swift and Company, for preparing and interpreting the microscopic sections.

TABLE 1

Cooking Factors, Judges' Scores, and Soluble Nitrogen for Veal Cooked to Different Internal Temperatures

Factors	Uncooked roasts	Internal temperature of cooked roasts				F-value
		71°C. (160°F.)	77°C. (170°F.)	82°C. (180°F.)	88°C. (190°F.)	
SMALL CARCASS						
Average weight of roasts (gm.)	.....	183.0	183.0	176.0	185.0	.....
Cooking losses (pct.)						
Total	.....	13.1	22.5	29.1	36.8	.....
Drip	.....	1.0	1.2	0.9	0.7	.....
Evaporation	.....	12.1	21.3	28.3	36.1	.....
Cooking time (min./lb.)	.....	120.0	178.0	235.0	307.0	.....
Judges' scores (av.)						
Color	.....	2.4	3.2	3.4	3.8	18.80[1]
Flavor	.....	2.5	3.1	3.6	3.9	30.30[1]
Texture	.....	2.2	2.9	3.1	3.7	22.00[1]
Juiciness	.....	4.3	3.8	3.2	2.6	63.40[1]
Tenderness	.....	3.2	3.4	3.5	3.8	5.40[1]
Soluble N/gm. total N in meat (eg.)	10.52	9.84	8.77	8.99	8.12	14.30[1,3]
Soluble N from enzyme action/gm. total N in meat (eg.)	3.30	6.13	7.44	4.92	5.31	2.05[4]
MEDIUM CARCASS						
Average weight of roasts (gm.)	.....	412.0	389.0	367.0	403.0	.....
Cooking losses (pct.)						
Total	.....	13.9	20.8	30.1	35.6	.....
Drip	.....	1.1	1.7	1.2	1.3	.....
Evaporation	.....	12.7	19.1	28.9	34.3	.....
Cooking time (min./lb.)	.....	87.0	113.0	169.0	185.0	.....
Judges' scores (av.)						
Color	.....	2.7	3.2	3.1	3.3	3.33[2]
Flavor	.....	2.3	2.9	3.0	3.7	20.70[1]
Texture	.....	2.3	2.9	3.1	3.4	9.95[1]
Juiciness	.....	4.0	3.6	3.1	2.5	32.20[1]
Tenderness	.....	2.8	3.0	3.0	3.6	5.52[1]
Soluble N/gm. total N in meat (eg.)	13.09	9.94	9.32	9.40	8.95	189.00[1,3]
Soluble N from enzyme action/gm. total N in meat (eg.)	3.33	6.60	6.62	5.08	5.76	0.54[4]
LARGE CARCASS						
Average weight of roasts (gm.)	.....	534.0	544.0	539.0	511.0	.....
Cooking losses (pct.)						
Total	.....	15.2	23.7	31.5	37.2	.....
Drip	.....	2.1	3.2	2.0	2.0	.....
Evaporation	.....	13.1	20.5	29.5	35.2	.....
Cooking time (min./lb.)	.....	81.0	107.0	141.0	174.0	.....
Judges' scores (av.)						
Color	.....	2.6	3.3	3.5	3.5	16.50[1]
Flavor	.....	2.8	3.4	3.7	4.1	34.30[1]
Texture	.....	2.5	3.3	3.4	3.4	14.97[1]
Juiciness	.....	4.1	3.3	2.9	2.2	40.20[1]
Tenderness	.....	2.8	3.1	3.3	3.4	4.15[2]
Soluble N/gm. total N in meat (eg.)	9.64	8.55	7.33	6.75	6.16	16.90[1,3]
Soluble N from enzyme action/gm. total N in meat (eg.)	1.83	3.01	4.22	3.05	2.34	1.42[4]

[1] Highly significant. [2] Significant. [3] Includes data on raw meat. [4] Does not include data on raw meat.

Digestion: The cooked samples were more readily digested *in vitro* than were the raw ones, but there was no indication that the relative digestibility of the cooked samples varied consistently with the internal temperature.

The figures on the amount of soluble nitrogen in the meat itself (without enzyme treatment) were investigated with the idea that perhaps the breakdown of collagen to gelatin might show up as an increase in soluble nitrogen with cooking. However, the trend was just the opposite, a steady decrease being obtained in most cases from the raw through the range of increasing internal temperatures. This decrease probably was caused by several factors, such as the loss of soluble nitrogenous material in the drippings and also the binding of soluble nitrogen compounds by the coagulation of the tissue proteins.

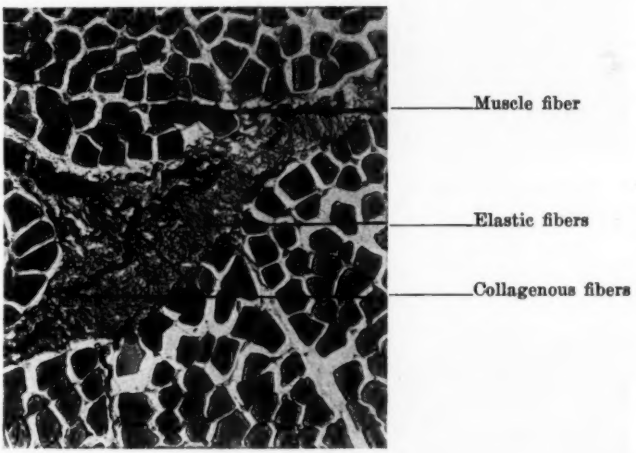

FIG. 2. Uncooked semitendinosus muscle showing collagenous fibers (200 ×).

Color: The raw samples varied in hue from red to yellow red, were about in the middle (4-6) of the value scale, and were quite intense (6-10) in chroma. With cooking, the hue shifted toward yellow, the value decreased (grayness increased), and the chroma remained about the same. The change in value, particularly, was progressive with increasing internal temperature.

Histological Changes: Two changes in the connective tissue were observed in the slides of the cooked veal. The first was an apparent swelling or "spreading" of the collagenous fibers observed particularly in the semitendinosus muscle. These fibers were narrow and compact before cooking (Fig. 2). When the meat was cooked to a temperature of 88°C. (190°F.) these fibers showed considerable "spreading" (Fig. 3). There was no evidence of change in the elastic connective tissue. The other change was in the fine connective tissue sheath or endomysium which surrounds each individual muscle fiber. In the raw muscles, the endomysium appears to be continuous and intact (Fig. 4). During cooking,

FIG. 3. Semitendinosus muscle cooked to 88°C.(190°F.) showing "spreading" of collagenous fibers (200 ×).

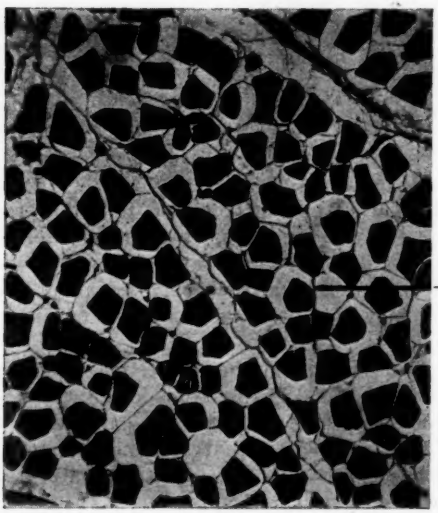

FIG. 4. Uncooked semitendinosus muscle showing intact endomysium (200 ×).

the endomysium tends to become disrupted (Fig. 5). The breakdown of this connective tissue framework loosened the individual muscle fibers and made the cooked samples more difficult to section. These two changes, the "spreading" of the collagenous fibers and the breakdown of the endomysium surrounding the individual fibers, might be expected to contribute to the increase in tenderness that occurs in some pieces of meat under some cooking conditions and procedures.

FIG. 5. Semitendinosus muscle cooked to 88°C.(190°F.) illustrating how cooking tends to disrupt endomysium (200 ×).

There was also some evidence that, during cooking, the sarcolemma of the muscle fibers became disrupted. In raw muscles where the sarcolemma was intact, the edges of the fibers had a straight smooth appearance (Fig. 4). In cooked meat these edges tended to become irregular and rough (Fig. 5). The slides also showed change in the staining reaction of the tissues with cooking, the connective tissue of the cooked samples staining less intensely.

Effect of Size of Animal: The average values for cooking factors, judges' scores, soluble nitrogen, and increase in soluble nitrogen from enzyme activity for the three sizes of animals used are shown (Table 2).

The losses did not differ greatly between the small and medium sizes. The increased total losses and drippings for the large size were a reflection of the much larger amount of fat on these cuts. The cooking time in minutes per pound decreased with increase in size, since the weights of the cuts used increased much more in proportion than did the distance which the heat had to penetrate to the centers of the roasts.

The roasts from the small size were quite pink at the lower temperatures, while those from the medium and large sizes developed a grayish-

brown color at the higher internal temperatures. The judges considered both the pink and the brown colors undesirable. Therefore, these two effects tended to balance each other, so that the average score for color did not vary significantly between the sizes. This is perhaps better illustrated by comparison of the figures for color (Table 1) where the small size had the lowest score of the three sizes at 71°C.(160°F.) but the highest score at 88°C.(190°F.).

TABLE 2

Cooking Factors, Judges' Scores, Soluble Nitrogen, and Soluble Nitrogen From Enzyme Action in Three Sizes of Veal

Factors	Small carcass	Medium carcass	Large carcass	F-value
Cooking losses (pct.)				
Total	25.4	25.1	26.9	.....
Drip	0.95	1.3	2.3	.....
Evaporation	24.5	23.8	24.6	.....
Cooking time (min./lb.)	210.0	139.0	126.0	.....
Judges' scores (av.)				
Color	3.2	3.1	3.2	1.57
Flavor	3.3	3.0	3.5	14.87[1]
Texture	2.9	2.8	3.1	5.40[1]
Juiciness	3.5	3.3	3.1	11.50[1]
Tenderness	3.5	3.1	3.1	8.29[1]
Soluble N/gm. total N in meat (cg.)	9.26	10.14	7.68	11.90[1]
Soluble N from enzyme action/gm. total N in meat (cg.)	5.95	5.91	3.15	14.01[1]

[1] Highly significant.

The roasts from the largest animals were preferred for flavor and texture, but the preference was not progressive with size, as the roasts from the smallest animals rated second. The roasts from the smallest animals were juiciest, with the middle size second, and the large size lowest. The smallest animals also gave the most tender roasts. These results were rather surprising since fat, especially intramuscular fat, is generally considered to increase the juiciness and tenderness of meat. The roasts from the small animals had no apparent intramuscular fat and almost no intermuscular fat, while the roasts from the large animals had a good deal of intermuscular and usually some intramuscular fat. As far as juiciness was concerned, perhaps the change in the distribution of water in the muscles was sufficient to overbalance the increase in fat, as Lowry, *et al.* (1942) and Yannet and Darrow (1938) have shown that while there is little change in the percentage of total water in the muscles with growth, there is a decided shift in the location of the fluid from the extracellular to the intracellular compartment.

Both the original soluble nitrogen and the increase due to enzyme activity were about the same for the small and medium-sized animals, but decreased for the large animals.

The samples from the large animals were somewhat more yellow in hue than those from the small ones. Also, both the grayness and the intensity

increased with increasing size (value decreased, chroma increased). These changes were shown in both the raw and the cooked samples.

SUMMARY

In a study of the effect of increasing the internal temperature of veal roasts obtained from small, medium, and large calves the following results were obtained:

The cooking losses and cooking time and the scores for color, flavor, texture, and tenderness of the meat all increased while the juiciness decreased, with increasing internal temperature.

The cooking losses increased slightly with size of animal, while the cooking time in minutes per pound decreased. The roasts from the large animals gave the best results for flavor and texture, while those from the small animals were juiciest and most tender. In the judges' scores for texture, tenderness, and color, the extent of the variation induced by increase in internal temperature was less for roasts from large animals than for roasts from small animals.

The comparative digestibility *in vitro* did not increase with increasing internal temperature. However, all the cooked samples were more readily digested than were the raw ones. The roasts from the large animals had less soluble nitrogen and were less readily digested *in vitro* than those from the other two sizes.

The changes in microscopic appearance brought about by cooking were (1) swelling of the collagenous fibers observed in the semitendinosus muscle, (2) partial breakdown of the connective tissue reticulum surrounding the muscle fibers, and (3) changes in the staining properties of the sections.

In general, of the temperatures studied, the judges thought that 82°C. (180°F.) gave the most satisfactory results, particularly for the roasts from the smallest animals.

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STUDIES ON VEAL. II. VARIATIONS BETWEEN SOME MUSCLES OF THE HIND QUARTER

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The variation between different muscles of the same animal and between the same muscles from different animals, is of great importance in planning experiments. During a study of the effect of different internal temperatures on the palatability of veal it was possible to collect some data on this problem.

The materials and methods used in this study have already been discussed by the writers in the first paper which immediately precedes this one.

RESULTS

Differences Between Muscles: The data on weight, cooking factors, and judging factors for the various muscles are given (Table 1). It was obvious that the muscles differed considerably among themselves, which made it inadvisable to disregard them as one of the sources of variation in analyzing the data.

Cooking Factors: The cooking time tended to vary inversely with the average weight of the various cuts. The short cooking time in relation to weight of the biceps femoris was partly due to its shape. The biceps is a wide, flat muscle, whereas all the other muscles approach more nearly a round or square cross section. The variations in the cooking losses indicated that the losses were influenced by both the weight and the cooking time. In addition, such variables as amount and distribution of fat undoubtedly had some influence on the extent of the losses and on the relative distribution of the total losses between drip and evaporation.

Judges' Scores: The semitendinosus and biceps femoris were the juiciest muscles, while the semitendinosus, gluteus medius, and rectus femoris were the most tender. The semimembranosus was the driest and one of the toughest of the muscles used. For the other judging factors of color, flavor, and texture the semimembranosus, gluteus medius, and rectus femoris were generally the best.

Histological Studies: Since there was a great deal of variation in the amount of connective tissue and in the size of the fasciculi, it was difficult to group the muscles according to microscopic appearance.

The semitendinosus (Fig. 1) had much connective tissue, and the perimysium divided the muscle into numerous medium-sized fasciculi. Elastic connective tissue was very abundant in the larger connective tissue areas.

The semimembranosus (Fig. 2) had a smaller amount of connective tissue which consisted almost entirely of collagenous fibers.

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The adductor had a large amount of connective tissue composed both of elastic and collagenous fibers. The fasciculi were quite uniform in size but not all parallel, some running at angles to others. In general, little fat was observed in the veal muscles, but the adductor contained some of this tissue.

The perimysium of the biceps femoris (Fig. 3) consisted of thick masses of connective tissue in which were found many collagenous fibers and few elastic fibers. Most of the fasciculi were parallel.

TABLE 1

Differences Between the Various Muscles Employed

Factors	Semi-tendi-nosus	Semi-mem-branosus	Biceps femoris	Gastroc-nemius-vastus lateralis	Gluteus medius-rectus femoris	Adductor-vastus medialis	F-value
SMALL CARCASS							
Cooking losses (pct.).							
Total	14.8	33.0	27.0	24.3	24.7	28.4	....
Drip	0.0	2.0	1.1	0.7	1.0	1.1	....
Evaporation	14.8	31.0	26.0	23.7	23.7	27.3	....
Cooking time (min./lb.)	236.0	213.0	182.0	193.0	190.0	246.0	....
Av. weight of roasts (gm.)	86.0	197.0	253.0	210.0	209.0	136.0	....
Judges' scores (av.)							
Color	3.0	3.7	3.4	3.1	3.2	2.8	4.4[1]
Flavor	3.1	3.8	3.3	2.9	3.4	3.0	5.6[1]
Texture	2.8	3.5	3.1	2.6	3.0	2.6	4.8[1]
Juiciness	3.9	3.0	3.6	3.5	3.4	3.4	6.3[1]
Tenderness	3.6	3.5	3.5	3.2	3.7	3.2	3.2[2]
MEDIUM CARCASS							
Cooking losses (pct.)							
Total	15.6	30.2	26.4	24.3	24.2	29.6	....
Drip	0.6	2.4	1.7	1.0	1.3	1.1	....
Evaporation	15.0	27.8	24.7	23.3	22.9	28.6	....
Cooking time (min./lb.)	132.0	153.0	110.0	133.0	117.0	188.0	....
Av. weight of roasts (gm.)	198.0	454.0	531.0	432.0	443.0	298.0	....
Judges' scores (av.)							
Color	2.9	3.3	3.3	2.7	3.0	3.2	2.1
Flavor	2.6	3.1	3.2	2.8	3.2	3.0	2.3
Texture	2.4	3.1	3.1	2.6	2.9	3.1	3.5[2]
Juiciness	3.9	2.7	3.2	3.4	3.4	3.2	7.7[1]
Tenderness	3.2	3.6	3.1	2.8	3.0	3.1	2.1
LARGE CARCASS							
Cooking losses (pct.)							
Total	20.0	30.2	27.7	28.0	26.3	29.3	....
Drip	1.8	3.3	3.5	1.4	2.0	1.9	....
Evaporation	18.2	26.8	24.3	26.5	24.3	27.4	....
Cooking time (min./lb.)	135.0	122.0	92.0	121.0	131.0	154.0	....
Av. weight of roasts (gm.)	196.0	656.0	809.0	584.0	457.0	392.0	....
Judges' scores (av.)							
Color	2.9	3.4	3.4	3.3	3.4	3.0	3.0[2]
Flavor	3.4	3.5	3.6	3.4	3.7	3.4	1.2
Texture	3.1	3.3	3.2	3.0	3.2	3.0	1.0
Juiciness	3.7	2.5	3.2	3.1	3.1	3.0	6.2[1]
Tenderness	3.4	2.5	3.2	3.1	3.5	3.2	4.5[1]

[1] Highly significant. [2] Significant.

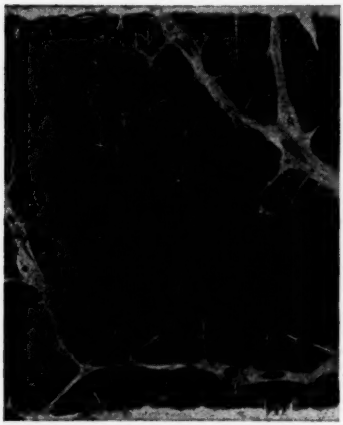

FIG. 1. Semitendinosus cooked to 71°C. (160°F.), showing medium-sized fasciculi and abundant connective tissue (6 ×).

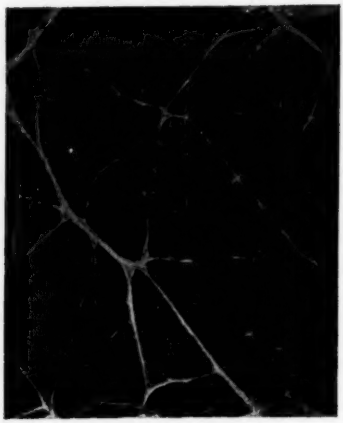

FIG. 2. Semimembranosus cooked to 71°C.(160°F.), showing larger fasciculi and a little connective tissue (6 ×).

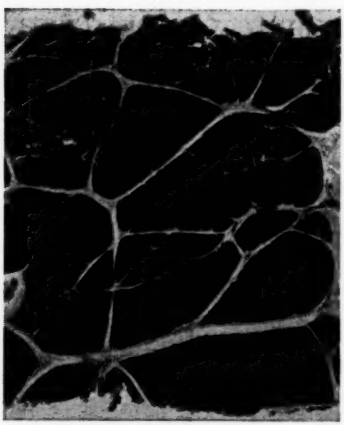

FIG. 3. Biceps femoris cooked to 71°C. (160°F.), showing heavy strands of connective tissue separating fasciculi (6 ×).

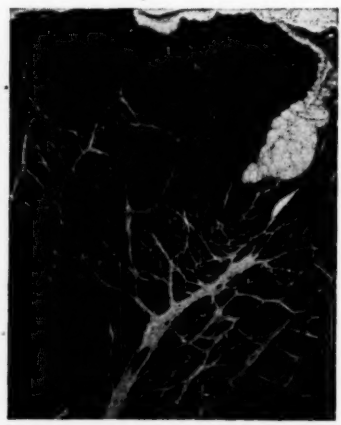

FIG. 4. Gastrocnemius cooked to 71°C. (160°F.), showing heavy tendinous mass in upper right hand corner. Since such masses are largely collagenous, they are usually softened by cooking (6 ×).

TABLE 2

Differences Between Animals Within Size Groups

Factor	Small carcass			Medium carcass			Large carcass		
	1	2	F-value	1	2	F-value	1	2	F-value
Cooking losses (pct.)									
Total	24.2	26.5	.....	25.3	24.8	.....	26.8	27.0	.....
Drip	0.9	1.1	.....	1.5	1.1	.....	2.0	2.7	.....
Evaporation	23.3	25.4	.....	23.8	23.7	.....	24.8	24.3	.....
Cooking time (min./lb.)	231.0	189.0	.....	134.0	143.0	.....	135.0	117.0	.....
Judges' scores (av.)									
Color	3.2	3.2	<1	3.1	3.0	<1	3.1	3.4	6.83[2]
Flavor	3.3	3.2	<1	3.2	2.8	6.90[1]	3.5	3.5	<1
Texture	3.0	2.9	<1	2.9	2.8	<1	3.2	3.1	<1
Juiciness	3.5	3.4	<1	3.3	3.3	<1	3.1	3.1	<1
Tenderness	3.4	3.5	2.54	3.1	3.1	<1	3.1	3.2	<1
Soluble N/gm. total N in meat (eg.)	7.73	10.78	13.60[2]	9.99	10.29	5.50	7.47	7.90	1.45
Soluble N from enzyme action/gm. total N in meat (eg.)	5.15	6.75	4.21	6.50	5.68	<1	2.25	4.05	7.56

[1] Significant. [2] Highly significant.

The vastus lateralis contained a large amount of connective tissue, having many collagenous fibers and a medium number of elastic fibers. Fatty tissue was found in this muscle. The fasciculi were not all parallel.

The rectus femoris had much connective tissue which subdivided the muscle into small fasciculi, most of which ran parallel to the length of the muscle. Very little elastic tissue but considerable collagenous tissue was present.

The perimysium of the vastus medialis was very branching and showed a medium amount of elastic and collagenous connective tissue. The gastrocnemius (Fig. 4) had a large amount of collagenous but very few elastic fibers. The gluteus medius contained a moderate amount of connective tissue. Very little elastic tissue was present.

Collagenous fibers were present in all muscles studied. However, there was considerable variation in the amount of elastic tissue present. The semitendinosus had a great deal of elastic connective tissue; the adductor, biceps femoris, vastus lateralis, and vastus medialis had moderate amounts; while the semimembranosus, rectus femoris, gastrocnemius, and gluteus medius had little elastic tissue.

Difference Between Animals Within Sizes: In general, there was very little significant variation between the two animals used as checks within each size classification, as can be seen from the data (Table 2).

The only significant variations in the judges' scores were between the two large animals for color and between the two medium animals for flavor. These differences may have been due to the fact that the two animals in each class were not of the same breed.

We were unable to account for the decided difference between the soluble nitrogen contents of the muscles of the two small animals. The only known difference between the two was sex, the female having the higher nitrogen content.

SUMMARY

In reviewing data on cooking factors and judges' scores for roast veal, from the standpoint of differences between muscles of the same animal and differences between the two animals used as checks in each size class, the following results were observed:

The variations in cooking losses and in palatability factors among the different muscles were quite large. These differences usually accounted for a significant portion of the total variation observed.

In most cases results on the cooking and judging factors for the two animals within each weight class were very similar.

ACKNOWLEDGMENT

We are indebted to Dr. E. J. Strandine, Research Laboratories, Swift and Company, for preparing and interpreting the microscopic sections.

RELATIONSHIPS OF CHEMICAL PROPERTIES OF SOILS NEAR MIDLAND, DOUGLAS COUNTY, KANSAS, TO YIELD OF RYE HARVESTED AT JOINTING STAGE[1]

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(Received for publication, April 10, 1945)

The chemical properties of a group of soils near Midland, Douglas County, Kansas, have been described by Wynd and Romig (1943), and the relationships of these properties to the yields of dry matter of oats cut at the jointing stage have been reported by Wynd and Noggle (1945). The data discussed in the latter paper, obtained during the autumn of 1940, indicated that several chemical characteristics of the surface soil, especially the amount of total replaceable bases and other characteristics which are related to it, were of great importance in determining the yield of dry matter of oats cut at the jointing stage.

The extensive use of dehydrated, immature cereal grasses as a source of protein and vitamins for human consumption and for stock feeds suggests the importance of a more thorough knowledge of the effects of the recognizable soil properties on the growth and composition of these species. The present study was carried out to determine the applicability of the conclusions, arrived at previously, to the growth of rye at a different season of the year and on a different group of soils.

The eight soils included in the present study fall into the following classes on the basis of the characteristics of their surface soils described by Wynd and Romig (1943). It is important that these characteristics be borne in mind when evaluating certain relationships presented in the figures.

Total replaceable bases small (10.3 to 14.8 me.)
Fields 9, 10, 19B, 20, 22
Points 1, 2, 3, 4, 5

Total replaceable bases large (18.0 to 23.7 me.)

Soils not calcareous. pH less than 7.0. Base saturation less than 100 per cent.
Fields 15A, 15B
Points 7, 8

Soils calcareous. pH more than 7.0. Base saturation greater than 100 per cent.
Field 11
Point 6

[1] The expenses incurred during the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

The crops were harvested at, or just preceding, the jointing stage and were dried in an Arnold hot-air dehydrator. The individual fields were from 25 to 40 acres in extent and their entire yields were used in calculating the yields per acre; the data from eight fields are presented (Table 1). The point numbers serve to identify in the figures the data from individual fields. M.S.C. file numbers refer to the original samples permanently on file in the Laboratory of Plant Physiology at Michigan State College. The file numbers of the soil samples will enable the reader to locate the chemical data for each soil in the tables appearing in the earlier paper by Wynd and Romig (1943).

TABLE 1

Yields of Dry Matter of Rye, Cut at Jointing Stage, Grown on Various Soils in Vicinity of Midland, Kansas

M.S.C. file No.		Field No.	Point No.	Date harvested	Yield per acre
Soil	Rye				
					lb.
286	332	9	1	Apr. 10, 1941	144
285	334	10	2	Apr. 11, 1921	221
288	335	20	3	Apr. 12, 1941	379
284	336	19B	4	Apr. 13, 1941	500
287	337	22	5	Apr. 14, 1941	298
291	338	11	6	Apr. 17, 1941	947
289	339	15A	7	Apr. 20, 1941	1430
290	340	15B	8	Apr. 20, 1941	1950

DISCUSSION

The base-exchange capacity is shown (Fig. 1) to be related to the yield of rye cut at the jointing stage. Fields 9, 10, 19B, 20, and 22 (Points 1-5, inclusive) vary but slightly in their base-exchange capacity, and these variations have but a small effect on the yield, but when the values are greater than about 12 milliequivalents per 100 grams, the effect on the yield is great.

The amount of total replaceable bases is shown (Fig. 2) to be particularly closely associated with the yield. Even comparatively small variations in the amount of replaceable bases exert noticeable effects.

The relationship of the degree of base saturation is not consistent. It appears (Fig. 3) that growth on Fields 9, 10, 11, 19B, 20, and 22 (Points 1-6, inclusive) respond favorably to higher degrees of base saturation, while Fields 15A and 15B (Points 7 and 8) do not. This situation probably is due to the increasing amount of replaceable bases which are responsible for the degree of saturation in the first group. The yields from Fields 15A and 15B (Points 7 and 8) are related to the amount of bases which they contain, regardless of the effect of these bases on the degree of base saturation. The degree of base saturation, *per se*, has therefore little or no intrinsic influence on the growth of rye within the limits found in the soils included in the present study.

The apparent relationship, when soils of approximately equal base exchange capacity are considered, depends on the fact that the degree of base saturation is proportionate to the amount of total replaceable bases present.

The influence of pH is identical to that of the degree of base saturation. The pH values likewise appear to be related to the yields from soils of low base-exchange capacity, but the yields appear independent of pH when the base-exchange capacity is greater. This condition is dependent

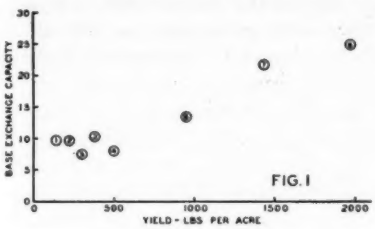


FIG. 1

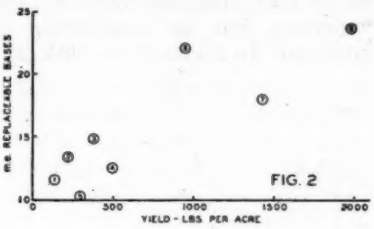


FIG. 2

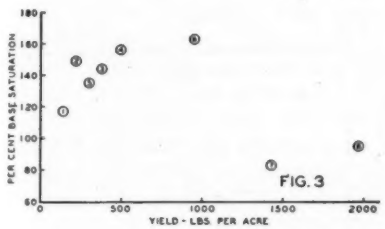


FIG. 3

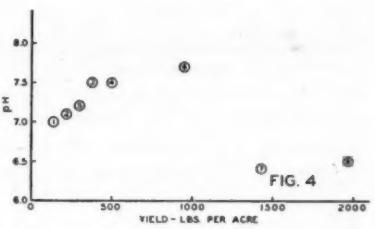


FIG. 4

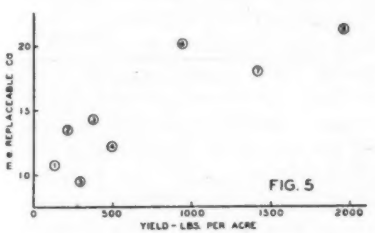


FIG. 5

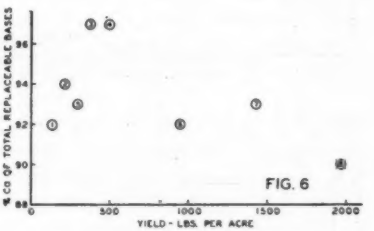


FIG. 6

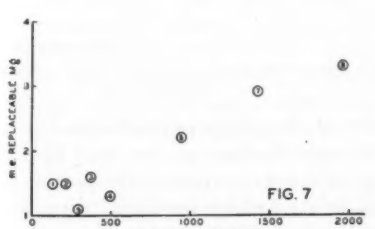


FIG. 7

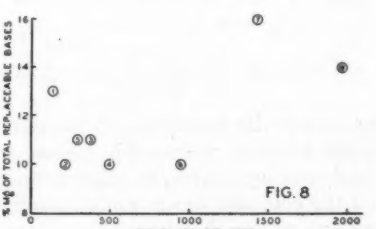
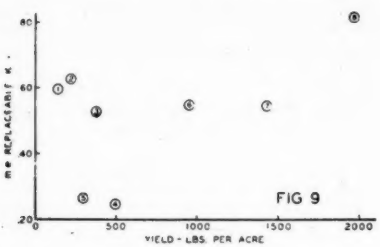


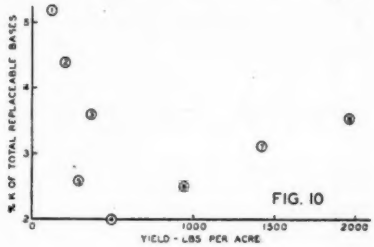
FIG. 8

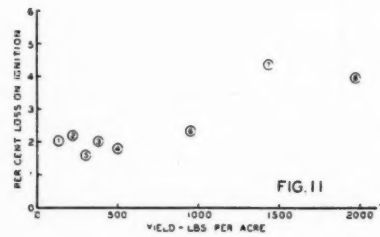
on the fact that the growth of rye was dependent on the total replaceable bases, while pH is only a reflection of the degree of base saturation. The amount of calcium carbonate parallels replaceable bases, as determined in an ammonium acetate leachate in fields of low base-exchange capacity, and therefore, indirectly, causes a positive relationship to appear between

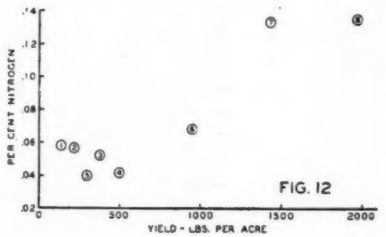
increasing values of carbonate and yield. On the other hand, this relationship is not apparent for the soils which have high base-exchange capacities and therefore large amounts of replaceable bases.

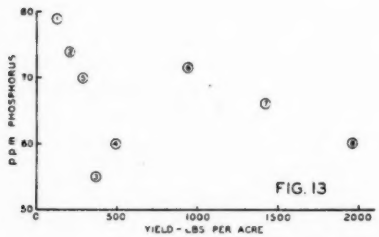
The amount of replaceable calcium is shown to be of great importance by the data presented (Fig. 5). Perhaps replaceable magnesium is equally important, but the relationship of replaceable potassium is difficult to interpret. In Fields 9, 10, 19B, 20, and 22 (Points 1-5, inclusive) it seems

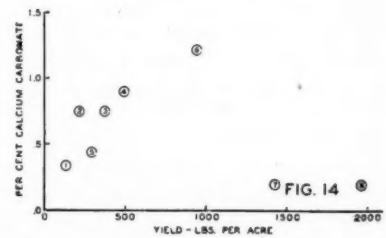












to be actually harmful, yet the best yields of the group were obtained from fields of high replaceable potassium. It may be that in the soils of low base-exchange capacity calcium and magnesium had replaced the potassium on the colloids, which would result in its being lost by leaching. Since the growth was so closely related to the calcium and magnesium, perhaps these ions were the limiting factors even though their presence may have lessened the amount of potassium remaining on the colloids.

Relation of the ratios of individual replaceable bases to the total amount of replaceable bases is shown (Figs, 6, 8, and 10). Wynd and Noggle (1945) have suggested that the numerical values of these ratios probably are without intrinsic physiological significance, within the range

of values studied, but more probably they represent the relative effects of the ions in the numerator as compared with the combined effects of those included in the denominator. For instance, if growth is increased by additional amounts of each base, then the greater growth will occur when the ratio of any one replaceable base to the total replaceable bases is smaller, because the effect of that base could not be as great as that of all bases.

The five soils containing low amounts of replaceable bases (Fig. 6, Points 1 to 5, inclusive) produced greater growth of rye as the amount of calcium relative to the total bases increased. It is probable that in these relatively poor soils, the calcium is relatively more important than magnesium or potassium. Soils represented by Points 6, 7, and 8, which contain more adequate supplies of bases, do not show this relationship of calcium. Data presented (Fig. 8) support this theory of the importance of calcium in the poorer soils, since the ratio of magnesium to the total bases, represented by Points 1 to 6, inclusive, diminishes as the yield increases. More impressive evidence is presented (Points 1 to 5, inclusive, Fig. 10) which shows an evident relationship between the amount of total bases as compared with that of potassium alone. It is impossible to describe fundamental relationships between the ratios of the amount of a single replaceable base to the amount of total bases from the present set of data.

Organic matter, represented by the loss on ignition, shows the same relationship to the yield of rye as the exchange capacity. The same is true of nitrogen. Since the organic matter largely determines the base-exchange capacity, and therefore indirectly the amount of total replaceable bases in the soil, and is itself nitrogenous, it would be difficult to decide which of these factors is of prime importance.

The amount of phosphorus soluble in weak acid appears to be inversely related to the yields from soils of low base-exchange capacity represented by Points 1 to 5, inclusive. Even the soils of high base-exchange capacity, represented by Points 6 to 8, inclusive, show this relationship. This might be due to a withdrawal by the crop of the comparatively small amounts of weak-acid-soluble phosphorus from soils which permit the greater growth. The problem of phosphorus availability is so intricate and uncertain that an interpretation of these data is impossible at the present time.

The data discussed above show that soils in the vicinity of Midland, Douglas County, Kansas, satisfactory for the growth of rye harvested at the jointing stage, are characterized by chemical characteristics having at least the following magnitudes: base-exchange capacity, 12 milliequivalents per 100 grams; total replaceable bases, 17 milliequivalents; replaceable calcium, 15 milliequivalents; replaceable magnesium, 2 milliequivalents; replaceable potassium, 0.50 milliequivalent; loss on ignition, 2.5 per cent; and nitrogen, 0.07 per cent. Probably the amount of total replaceable bases is the most important single property, although it is difficult to separate the effects of factors which necessarily vary simultaneously. The favorable limits to the chemical characteristics of soils are not absolute, but they depend on the rate of nutrient renewal in the soil and also on the losses owing to leaching and the removal of crops.

Soils in the vicinity of Midland which contain less than 15 milliequivalents of replaceable bases per 100 grams are unsatisfactory for the production of rye when cut at the jointing stage.

The responses of rye to soil properties are remarkably similar to those of oats described previously by Wynd and Noggle (1945). Any factor in the environment which is able to exert an observable effect on the growth of plants under the diverse conditions existing in the autumn and spring in eastern Kansas must be of profound importance since it must be dominant over the effects of other factors. When such an influence does appear, it is due to a factor of more physiological importance, therefore, than the mere numerical magnitude of its effect would suggest.

SUMMARY

The yield in dry matter of rye cut at the jointing stage grown on eight soils in the vicinity of Midland, Douglas County, Kansas, is related to certain chemical properties of the soil.

The total amount of replaceable bases is the most important chemical character related to the productivity.

The amounts of replaceable calcium and probably magnesium, also are related to productivity, especially in soils containing small amounts of replaceable bases.

Increasing base-exchange capacity, organic matter, carbonates, and pH appear to be related to the growth of rye, but only when they are associated with increasing amounts of replaceable bases.

Satisfactory soils in the vicinity of Midland for the production of dry matter of rye when harvested at the jointing stage are characterized by chemical properties having at least the following minimum values: base-exchange capacity, 12 milliequivalents per 100 grams; total replaceable bases, 17 milliequivalents; replaceable calcium, 15 milliequivalents; replaceable magnesium, 2 milliequivalents; replaceable potassium, 0.50 milliequivalent; loss on ignition, 2.5 per cent; and nitrogen, 0.07 per cent.

Soils containing less than 15 milliequivalents per 100 grams are unsatisfactory for the purpose described.

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RELATIONSHIPS OF CERTAIN CHEMICAL PROPERTIES OF SOIL NEAR MIDLAND, DOUGLAS COUNTY, KANSAS, TO ACCUMULATION OF PROTEIN IN RYE LEAVES HARVESTED AT JOINTING STAGE[1]

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A description of the chemical properties of a group of 10 soils in the vicinity of Midland, Douglas County, Kansas, has been published by Wynd and Romig (1943). The relationships of these properties to the growth of oats and to rye have been reported by Wynd and Noggle (1945a, 1946). The total amount of replaceable bases, as determined by titration of the ignited residue of the ammonium acetate leachate, was found to be the factor most closely related to growth of these species. The replaceable calcium was an especially important component of the replaceable bases. The influence of the nitrogen content of the soil was secondary to that of the replaceable bases.

One of the most significant results of the studies described above is the discovery of the similarity of the influence of the various chemical properties of the soil on the growth of both oats and rye, even though these species were grown during different seasons of the year and on a different group of soils.

Later, Wynd and Noggle (1945b) described the relationships between the chemical properties of these soils and the accumulation of protein in the leaves of oats. The most important factors affecting the percentage of protein in the leaves of oats were found to be the base-exchange capacity, loss on ignition, nitrogen, and replaceable magnesium. Since these factors in the soil were related quantitatively to each other, it was impossible to ascribe specific importance to them individually. It is reasonable to suppose, however, that the nitrogen content of the soil was the most important factor. The most important factors which influence the yield of protein in pounds per acre were found to be the total amounts of replaceable bases and of nitrogen in the soil. Usually, the yield in dry matter paralleled the yield in protein. The yield of protein closely followed the amount of nitrogen in the soils saturated with bases, but this was not true if the soils were unsaturated.

The successful utilization of dehydrated, immature cereal grasses as a source of protein and vitamins depends on the high concentration of these components in the tissue just before jointing begins. It is, therefore, particularly important to extend our knowledge of the soil factors which are associated with the accumulation of these substances in the immature vege-

[1] The expenses incurred in this study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

tative tissues. The purpose of the present study is to extend the previous observation to include the effects of the chemical properties of a different group of soils at a different season of the year on the accumulation of protein in the leaves of rye.

The eight soils included in the present study may be grouped into three classes on the basis of their chemical properties as described by Wynd and Romig (1943). Fields 9, 10, 19B, 20, and 22 (Points 1 to 5, inclusive) have a comparatively small amount of replaceable bases, while Fields 15A, 15B, and 11 (Points 7, 8, and 6, respectively) have comparatively large amounts of replaceable bases. Field 11 (Point 6) is a calcareous clay loam, while the other two members of the group having a high amount of replaceable bases are noncalcareous, dark-colored loams. These major differences have an important bearing on the interpretation of the data presented in the figures. Further details may be obtained in the papers by Wynd and Romig (1943) and Wynd and Noggle (1945a, 1945b, 1946).

TABLE 1

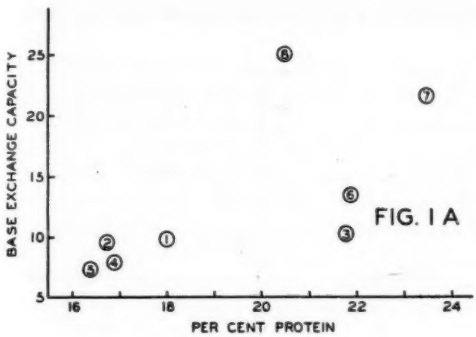
Accumulation of Protein in Rye Leaves

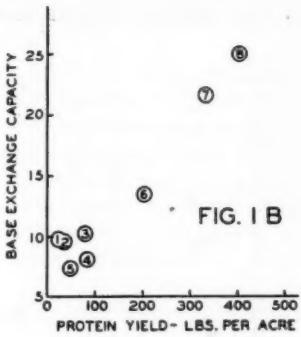
M.S.C. file No.		Field No.	Point No.	Date	Dry matter per acre	Nitrogen	N yield per acre	Crude protein (N × 6.25)	Protein yield per acre
Soil	Rye								
					lb.	*pct.*	*lb.*	*pct.*	*lb.*
286	332	9	1	Apr. 10, 1941	144	2.88	4.15	18.00	25.92
285	334	10	2	Apr. 11, 1941	221	2.68	5.92	16.75	37.02
288	335	20	3	Apr. 12, 1941	379	3.50	13.27	21.88	82.93
284	336	19B	4	Apr. 13, 1941	500	2.71	13.55	16.94	84.70
287	337	22	5	Apr. 14, 1941	298	2.62	7.81	16.94	48.81
291	338	11	6	Apr. 17, 1941	947	3.51	33.24	21.94	207.77
289	339	15A	7	Apr. 20, 1941	1430	3.77	53.91	23.56	336.91
290	340	15B	8	Apr. 20, 1941	1970	3.28	64.62	20.50	403.85

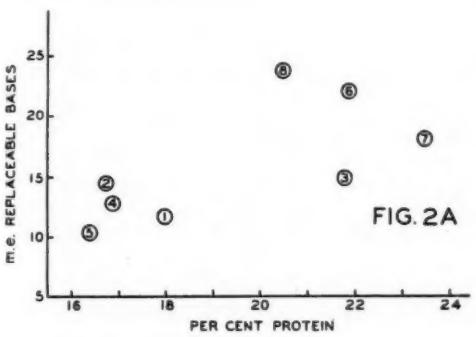
The determinations of protein were made on the same samples which furnished the data for the study of yield in dry matter previously reported by Wynd and Noggle (1946), and these determinations therefore show the effects of the same soils that were included in the former study. The total nitrogen was determined by the Kjeldahl method modified to include the nitrogen of nitrates as recommended by the Association of Official Agricultural Chemists (1940). The data (Table 1) are expressed as percentages of the oven-dry tissue and as yields in pounds per acre. The M.S.C. file numbers refer to the original samples permanently on file in the Laboratory of Plant Physiology at Michigan State College. The chemical data for each soil may be identified in the tables published by Wynd and Romig (1943) by referring to corresponding M.S.C. file numbers. The point numbers, which occur also in the figures, identify the soils from which each of the data was obtained.

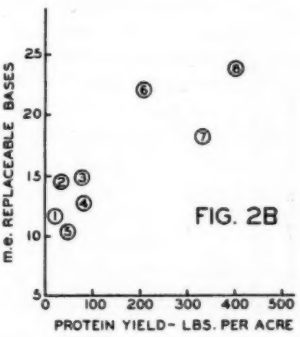
DISCUSSION

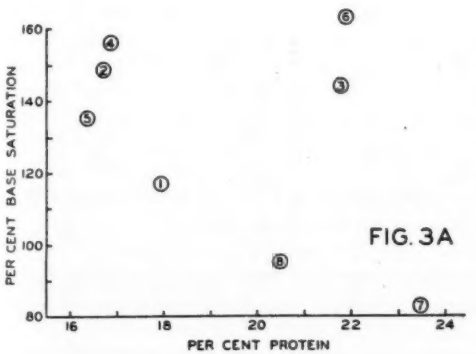
Examination of Fig. 1A shows that the base-exchange capacity of the soil is roughly related to the percentage of protein in the leaves of rye,

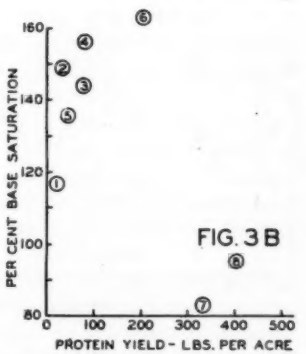

BASE EXCHANGE CAPACITY
PER CENT PROTEIN
FIG. 1 A


BASE EXCHANGE CAPACITY
PROTEIN YIELD - LBS. PER ACRE
FIG. 1 B

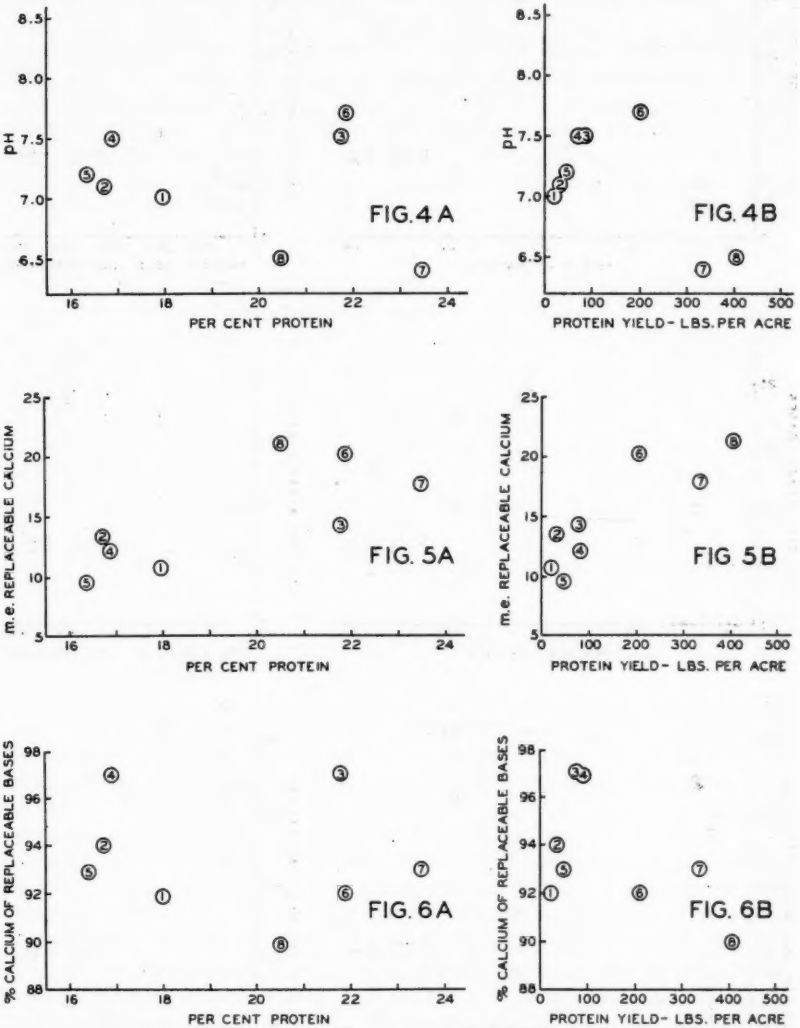

m.e. REPLACEABLE BASES
PER CENT PROTEIN
FIG. 2A


m.e. REPLACEABLE BASES
PROTEIN YIELD - LBS. PER ACRE
FIG. 2B


PER CENT BASE SATURATION
PER CENT PROTEIN
FIG. 3A


PER CENT BASE SATURATION
PROTEIN YIELD - LBS. PER ACRE
FIG. 3 B

although it is difficult to interpret the mechanism by which this property of the soil exerts its favorable influence. The distribution of the points in Fig. 11A, which relate the loss on ignition to the percentage of protein, is very similar to their distribution in Fig. 1A. A comparison of these



figures with Fig. 12A indicates that the organic matter is responsible for the major part of the base-exchange capacity and also for the nitrogen content of the soil. The base-exchange capacity determines to a very great extent the amount of replaceable bases retained in the soil. The amount of

replaceable bases thus appears to be the basis of the apparent importance of the base-exchange capacity. The relationship of the base-exchange capacity to the yield of protein in pounds per acre is particularly apparent since this factor is related to both the percentage of protein and the yield of dry matter.

The total amount of replaceable bases is shown by Fig. 2A to be related also to the percentage of protein in the tissue of rye leaves. The somewhat irregular distribution of the points indicates that this rather gross measurement of soil fertility is dependent on some accompanying factor for its apparent significance. As suggested above, this might be the nitrogenous nature of colloids responsible for the retention of the bases in the soil. The relationship of the total amount of replaceable bases to the yield of protein is very great since it is related to the yield of dry matter as well as to the percentage of protein in the tissue.

The degree of base saturation appears to be unrelated to the percentage of protein in the tissue. It is, however, related to the yield of protein because of the relationship which it bears to the yield of dry matter. Since the materials responsible for the major portion of the base-exchange capacity of the soils studied are nitrogenous, the degree of base saturation may be regarded as the ratio of the total replaceable bases to the nitrogen in the soil. The positive effect of the higher values of this ratio, as indicated by the distribution of Points 1 to 6, inclusive (Fig. 3B), shows that the growth of rye is more affected by higher amounts of replaceable bases than by greater amounts of nitrogen. The location of Points 7 and 8 supports this interpretation since the soils from which these tissues were obtained contained high amounts of replaceable bases, and therefore the growth of rye was limited by the nitrogen rather than by the bases in the soil.

The effect of pH on the percentage and total yield of protein resembles the effect of the degree of base saturation. This is to be expected, since the pH is a reflection of the degree of saturation.

The amount of replaceable calcium in the soil is shown (Fig. 5A) to be related to the percentage of protein in the tissue. The amount of replaceable calcium also is related to the yield of protein since the amount of replaceable calcium shows a positive relationship to the yield in dry matter as well as to the percentage of protein. Figure 7A shows that the amount of replaceable magnesium is also significantly related to the percentage and yield of protein. This situation is probably due to the fact that the amount of replaceable magnesium in the soils studied varies directly as the nitrogen content—Wynd and Romig (1943). The amount of exchangeable potassium is shown (Fig. 9A) to bear no relationship to the percentage of protein. The slight relationship of potassium to the yield of protein probably depends on its influence on the yield of dry matter.

A study of the effect of different ratios of the individual replaceable bases to the total amount of replaceable bases leads to interesting conclusions; for example, Fig. 6A shows that differences in the ratio of calcium to the total bases is of no definite importance, a fact which suggests that the effect of calcium is dominant to that of the other bases. The effect of changes in the magnitude of this ratio on yield of protein depends on their influence on the yield of dry matter. Points 1 to 5, inclusive, (Fig. 6B)

represent soils of low total replaceable bases. In these soils, calcium is the most important base, since higher ratios of calcium to the total bases are associated with high yields. Points 6 to 8, inclusive, represent soils containing large amounts of bases, and it appears that in such soils, bases other than calcium are of greater importance in the growth of rye.

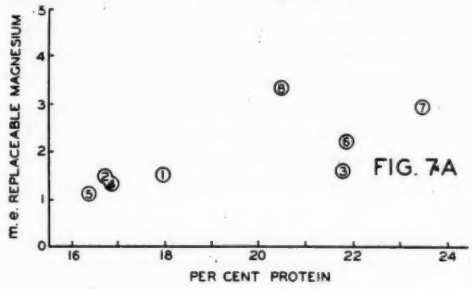


FIG. 7A

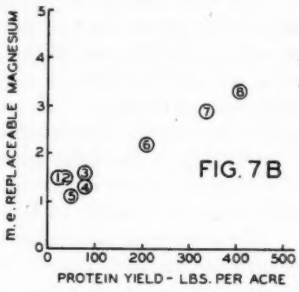


FIG. 7B

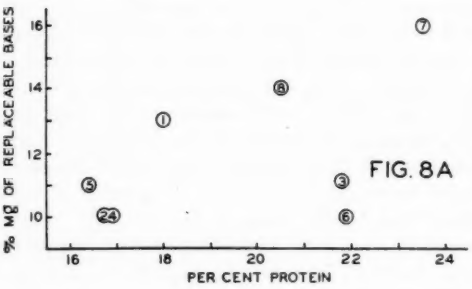


FIG. 8A

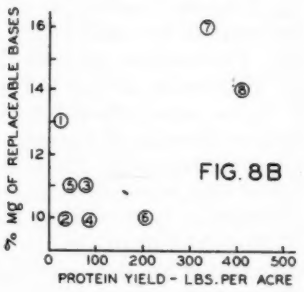


FIG. 8B

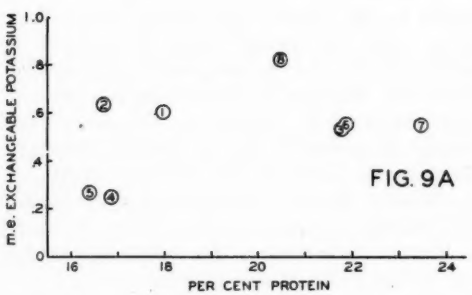


FIG. 9A

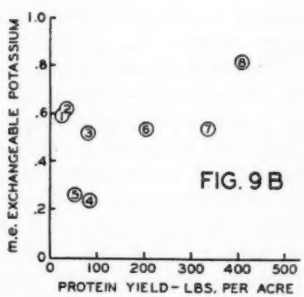
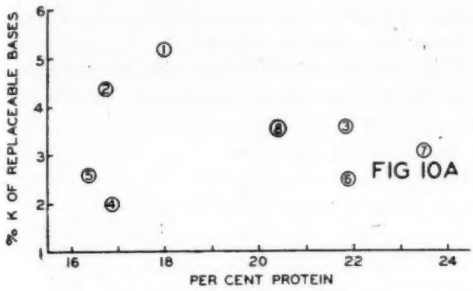
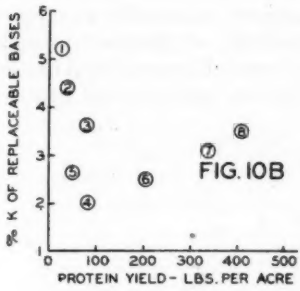


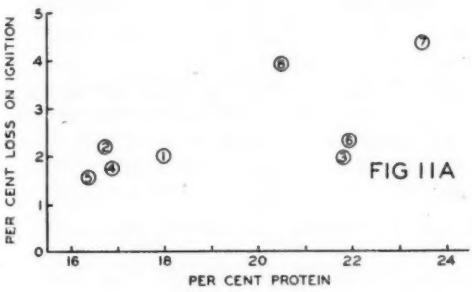
FIG. 9B

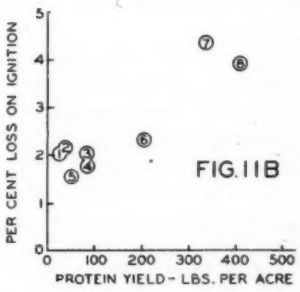
The higher ratios of magnesium to the total replaceable bases are generally associated with the higher percentages of protein, although the location of Points 3 and 6 (Fig. 9B) is irregular. Their effect on the yield of protein is even more difficult to determine. The soils represented by Points 7 and 8 are exceptionally rich in bases, and in these, the amount of magnesium appears to be unimportant. In the poor soils, represented by

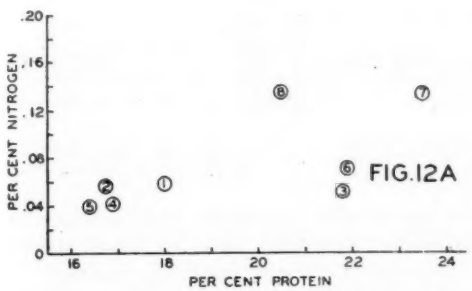
Points 1 to 6, inclusive, no definite relationship can be determined. Comparison of Fig. 8B with Fig. 8 in the authors' earlier report (1946) on the growth of rye shows that the relative distribution of the points is the same for the yields of protein and dry matter.

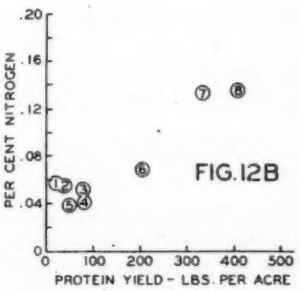






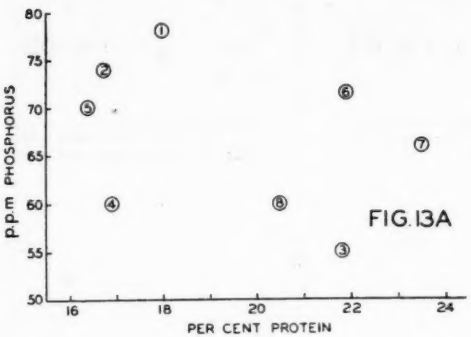


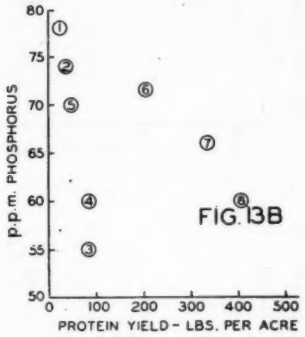


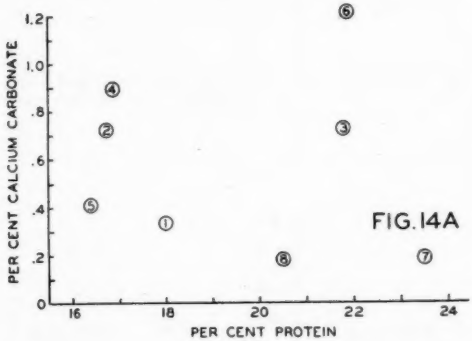


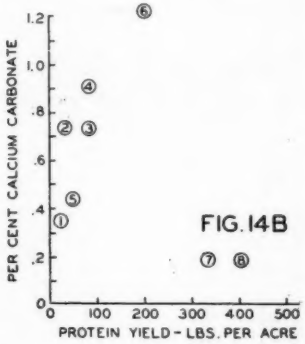
Lower ratios of potassium to the total bases are associated with the higher percentages of protein, which suggests that the effect of potassium is less than the combined effects of calcium and magnesium. This verifies the observation described above that the amounts of both calcium and magnesium were more important than was the amount of potassium. The yield of protein follows the yield of dry matter.

The authors stated, in a previous paper (1945b), that the percentage of protein in oats was closely related to the amount of nitrogen in the soil and also to replaceable magnesium, but since the differences in the amounts of each of these substances were always associated with similar differences in the other, it was impossible to separate their individual effects. It appears reasonable to suppose that nitrogen is the important factor of this complex of factors. A similar situation exists in respect to the response of rye. Figures 7A, 11A, and 12A, which relate replaceable magnesium, loss on ignition, and nitrogen to the percentage of protein in the tissue,









show an almost identical relative distribution of points. It is noteworthy that amounts of replaceable magnesium, more than the other bases, should vary so quantitatively with the amount of organic matter in the soil. A conventional interpretation would suggest that the amount of nitrogen in the soil is the more important factor.

Figures 11B and 12B show that the yield of protein is positively related to the amount of organic matter and nitrogen in soil since each of these soil properties favorably affects both the percentage of nitrogen in the tissue and the yield in dry matter.

The amount of phosphorus soluble in weak acid does not appear to be related to the percentage or to the yield of protein.

Calcium carbonate does not affect the percentage of protein in the leaves. It does affect the yield of protein especially in leaves produced by the poorer soils, since the yield of dry matter from these soils depends on the replaceable bases which accompany the carbonate.

The effects of the chemical properties of soil on the accumulation of protein in rye leaves is remarkably similar to their effects on protein accumulation in oat leaves reported previously by the authors (1945b), even though these species were grown during different seasons of the year on different groups of soils. It is apparent that any factor that can exert an observable effect under these diverse conditions must do so in competition with all other influences acting upon the plant. The effect of such a factor must be modified more or less by these other influences, and therefore its true physiological importance is greater than the measureable effects would indicate.

SUMMARY

The chemical properties of a group of eight soils near Midland, Douglas County, Kansas, were compared on the basis of their effects on the percentages and on the total yield of protein in rye leaves when harvested at the jointing stage.

The percentage of protein in the leaves was found to be associated primarily with the base-exchange capacity, loss on ignition, and nitrogen. The relationships of each of these factors to the percentage of protein could not be determined individually because they varied quantitatively with each other in the soils included in the present study. Nitrogen would be regarded conventionally as the most important of these factors.

The yield of protein in pounds per acre was related to the base-exchange capacity, total replaceable bases, replaceable calcium, replaceable magnesium, and the percentage loss on ignition. The most important of these probably was the amount of total replaceable bases. This was especially true of the base-saturated soils.

The yield of protein was related to the yield of dry matter rather than to the percentage of protein in the tissue.

Replaceable potassium and weak-acid-soluble phosphorus, within the limits of the values studied, were not related to accumulation of protein in rye leaves.

Satisfactory soils in the vicinity of Midland for the production of protein in rye leaves harvested at the jointing stage were characterized by chemical properties of at least the following magnitudes: base-exchange capacity, 13 milliequivalents per 100 grams; replaceable bases, 17 milliequivalents; replaceable calcium, 15 milliequivalents; replaceable magnesium, 2 milliequivalents; nitrogen, 0.06 per cent, and loss on ignition, 2.5 per cent.

Soils in the vicinity of Midland, having a base-exchange capacity less than 10 milliequivalents per 100 grams, replaceable bases less than 15 milliequivalents, and nitrogen less than 0.05 per cent were not satisfactory for the production of protein in rye leaves.

The effects of soil properties on the accumulation of protein in rye leaves grown in the spring of the year were remarkably similar to those

on oat leaves grown in the autumn. Rye appears able to thrive on poorer soils than oats. Seasonal differences may have enabled the rye leaves to accumulate protein on poorer soils than oats.

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RIBOFLAVIN CONTENT OF TROPICAL FOODS[1]

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A search of the literature reveals very little up-to-date information on the riboflavin content of tropical foods. In this paper we present the results of an investigation undertaken for the purpose of determining the riboflavin content of some cereals, fruits, and vegetables as well as other foodstuffs either produced or consumed in Puerto Rico and neighboring islands.

Sebrell (1942) summarized the clinical picture of ariboflavinosis in humans as follows: "(1) ocular lesions, consisting usually of vascularizing keratitis with photophobia, dimness of vision, severe injection of the vessels of the fornix and sclera, burning of the eyes, lacrimation, and in severe cases, opacities of the cornea; (2) oral lesions, consisting usually of linear fissures in the angles of the mouth, reddened, shiny denuded appearance of the lower lip, and flattening of the papillae of the tongue, which becomes magenta red in color; and (3) dermal lesions, consisting usually of seborrheic accumulations in the folds of the skin, especially in the nasolabial folds, around the eyelids, on the ears, and in some cases comedones and a sharkskin-appearing lesion on the nose and over the malar eminences. In some cases the seborrheic dermal lesions may be extensive and may involve other regions of the body."

The Committee of Food and Nutrition of the National Research Council (1943) has recommended a daily allowance of 2,700 micrograms of riboflavin for a moderately active man. On the basis of the present study, it would appear difficult to obtain the above amount of riboflavin in the average diet consumed by the majority of the population of Puerto Rico and neighboring islands, as this diet is principally composed of polished rice, dried beans, root crops, plantains and a few other vegetables, with a small amount of imported dried codfish and meats. Dairy products are conspicuously absent from this diet. According to the results obtained in the work reported here, all the major components of the above diet, excepting the beans, are poor sources of riboflavin. As far as Puerto Rico is concerned, skin lesions resembling those described above for ariboflavinosis

[1] Published with the approval of the director of the School of Tropical Medicine and the director of the Agricultural Experiment Station of the University of Puerto Rico. A co-operative project.

TABLE 1

Riboflavin Content of Tropical Foods

Material	Common name in Spanish	Description of sample	Number of samples tested	Micrograms per 100 grams of fresh edible portion		
				Max.	Min.	Av.
Vegetable products:						
Cereals and cereal products:						
Corn (*Zea mays* L.)	Maiz	Fresh from the cob	5	120	110	118
Corn meal (*Zea mays* L.)	Harina de maiz	Native	6	70	60	66
"Gofio"	Gofio	Meal made of roasted corn	2	110	106	108
Rice (native) (*Oryza sativa* L.)	Arroz del país	Partly decorticated	2	67	49	58
Rice (polished) (*Oryza sativa* L.)	Arroz pulido	Imported	5	43	36	39
Rice (whole) (*Oryza sativa* L.)	Arroz	Imported	2	99	93	96
Fruits:						
Avocado (*Persea Persea* L.)	Aguacate	Ripe	5	204	143	160
Banana, var. gigante (*Musa sapientum* L.)	Guineo, var. gigante	Ripe	5	61	45	49
Banana, var. apple (*Musa sapientum* L.)	Guineo, var. manzana	Ripe	5	38	23	28
Banana, fig (*Musa sapientum* L.)	Guineo, niño	Ripe	5	68	51	63
Breadfruit (*Artocarpus communis* Forst.)	Panapén	Ripe	2	88	56	74
Cashew nut-fruit (*Anacardium occidentale* L.)	Pajuil	Ripe; pulp	5	52	34	54
Custard apple (*Annona reticulata* L.)	Corazón	Ripe	8	120	73	92
Genipa (*Genipa americana* L.)	Jagua	Ripe	12	411	136	275
Golden apple (*Spondias dulcis* Frost.)	Jobo de la India	Ripe	5	28	25	27
Grapefruit (*Citrus maxima* Burm.)	Toronja	Juice	14	26	13	18
Grapefruit (*Citrus maxima* Burm.)	Toronja	Whole edible part	5	45	37	42
Grape, sea (*Coccolobis uvifera* L.)	Uva de playa	Ripe	5	31	21	26
Guava (*Psidium guajava* L.)	Guayaba	(Obtained from Agr. Expt. Sta., Río Piedras)	....	....	....	....
Varieties:						
Dominica Blanca Agria	.....	Whole edible part; pulp and rind	3	65	52	59
Dominica Roja Agria	.....	Whole edible part; pulp and rind	3	49	46	47
Dominica Roja Dulce	.....	Whole edible part; pulp and rind	3	68	57	64
País Agria	.....	Whole edible part; pulp and rind	3	44	33	40
País Agria	.....	Ripe; rind	2	35	35	35
País Agria	.....	Ripe; pulp	2	36	34	35
País Dulce	.....	Whole edible part	3	45	38	41
Kanapy, bullace plum (*Melicocca bijuga* L.)	Quenepa	Ripe	10	76	31	51
Lemon (*Citrus limonia* Osbeck)	Limón	Juice of ripe fruit	5	73	47	59
Lime, sweet (*Citrus limetta* Risso)	Limón dulce	Juice of ripe fruit	5	15	12	13
Mamey (*Achras sapota* L.)	Mamey zapote	Ripe	5	42	37	39
Mango (*Mangifera indica* L.)	Mangó	Ripe; Mayaguez variety	7	75	63	73
Mesple (*Sapota achras* Mill)	Nispero	Ripe	4	37	25	31
Orange (*Citrus sinensis* L.)	China	Juice of ripe fruit	5	45	32	34
Papaw (*Carica papaya* L.)	Papaya	Ripe	6	39	28	33
"Pepino Angolo" (*Sicana odorifera* Vell.)	Pepino angolo	Ripe	5	51	45	50

Mesple (*Sapota achras* Mill.)	Níspero	Ripe	5	45	32	34
Orange (*Citrus sinensis* L.)	China	Juice of ripe fruit	6	39	28	33
Papaw (*Carica papaya* L.)	Papaya	Ripe				

"Pepino Angolo" (*Sicana odorifera* Vell.)	Pepino angolo	Ripe	5	71	45	57
Pineapple (*Ananas Ananas* L.)	Piña	Ripe	5	32	25	28
Pinguin, Maya Fruit (*Bromelia pinguin* L.)	Maya	Juice of ripe fruit	5	23	22	22
Pomegranate (*Punica granatum* L.)	Granada	Ripe	5	37	25	32
Soursop (*Annona muricata* L.)	Guanábana	Ripe	6	120	86	100
Star apple (*Chrysophyllum cainito* L.)	Caimito	Ripe	5	36	21	27
Strawberries (native) (*Rubus rosaefolius* Smith)	Fresas	Ripe	6	72	57	67
Tamarind (*Tamarindus indica* L.)	Tamarindo	Pulp surrounding seeds; ripe	5	180	96	143
West Indian locust (*Hymenaea courbaril* L.)	Algarrobo	Yellow powder surrounding seeds; ripe	10	233	95	176
Vegetables:						
Root crops:						
Arracacha (*Arracacia xanthorrhiza* Bancroft)	Apio	As sold in the market	5	66	49	57
Carrot (*Daucus carota* L.)	Zanahoria	As sold in the market	5	59	51	55
Cassava (*Manihot Manihot* L.)	Yuca	As sold in the market	5	33	29	30
Corn root, sweet (*Calathea allouia* Aubl.)	Lerenes	As sold in the market	5	43	29	36
Yam, white (*Dioscorea alata* L.)	Ñame blanco	As sold in the market	5	42	21	31
Yam, yellow (*Dioscorea cayenensis* Lam.)	Ñame amarillo	As sold in the market	3	44	34	39
Yautía, white (*Xanthosoma sagittifolium* L.)	Yautía blanca	As sold in the market	5	33	27	31
Yautía, yellow (*Xanthosoma atrovirens* C.)	Yautía amarilla	As sold in the market	5	47	35	44
Beans:						
Black beans (*Dolichos lablab* L.)	Chícharos	Dried; native	3	230	170	197
Cowpea (*Vigna unguiculata* L.)	Frijoles	Dried; native	5	220	190	210
Chick-pea (*Cicer arietinum* L.)	Garbanzos	Dried; imported	5	200	160	180
Navy beans (*Phaseolus vulgaris* L.)	Habichuelas blancas	Dried; imported	5	200	140	170
Pigeon pea (*Cajanus cajan* L.)	Gandules	Partly dried; native	5	270	210	230
Red kidney beans (*Phaseolus vulgaris* L.)	Habichuelas coloradas	Dried; imported	5	270	160	200
Rose beans (*Phaseolus vulgaris* L.)	Habichuelas rositas	Dried; imported	5	220	180	200
String beans (*Phaseolus vulgaris* L.)	Habichuelas tiernas	Fresh; native	6	170	110	130
Greens:						
Lettuce (*Lactuca sativa* L.)	Lechuga	Fresh	5	123	93	112
Okra (*Abelmoschus esculentus* L.)	Quimbombó	Fresh	5	76	68	73
Peppers, green (*Capsicum frutescens* L.)	Pimiento verde	Fresh	6	70	42	57
Peppers, sweet (*Capsicum frutescens* L.)	Ajíes dulces	Fresh	8	96	51	64
Water cress (*Roripa nasturtium aquaticum* L.)	Berros	Fresh	3	166	106	143
Seeds:						
Almond, tropical (*Terminalia catappa* L.)	Almendra tropical	Kernel	5	240	193	217
Breadfruit, seed (*Artocarpus integrifolia* L.)	Pepita de pan	Edible part	3	94	77	84
Coconut (*Cocos nucifera* L.)	Coco	Flesh from green coconut	2	33	32	33
Coconut (*Cocos nucifera* L.)	Coco	Water from green coconut	5	....	....	Less than 3
Sesame (*Sesamum orientale* L.)	Ajonjolí	Whole seed	2	219	214	217

TABLE 1 *(Concluded)*

Material	Common name in Spanish	Description of sample	Number of samples tested	Micrograms per 100 grams of fresh edible portion		
				Max.	Min.	Av.
Other types:						
Calabash (*Crescentia cujete* L.)	Higüera	Ripe	2	61	57	59
Chayote (*Sechium edule* Jacq.)	Chayote	Ripe	5	46	34	39
Eggplant (*Solanum melongena* L.)	Berenjena	Ripe	5	50	39	43
"Guarapo" (*Saccharum officinarum* L.)	Guarapo	Juice from cane	1	....	....	26
Onion (*Allium cepa* L.)	Cebolla	Whole	3	33	19	23
"Palmillo" (*Roystonea borinquena* Cook)	Palmillo	Pith of upper part of trunk	5	69	52	61
Plantain, green (*Musa paradisiaca* L.)	Plátano verde	As sold in the market	8	65	49	53
Plantain, yellow (*Musa paradisiaca* L.)	Plátono amarillo	As sold in the market	5	60	40	50
Squash (*Cucurbita moschata* Duch)	Calabaza	Ripe	6	65	34	43
Tomato (*Lycopersicon Lycopersicon* L.)	Tomate	Ripe	5	61	38	46
Animal products:						
Dairy products:						
Cheese (Puertorican)	Queso puertorriqueño	Resembles cottage cheese	6	361	307	341
Milk (cow's)	Leche de vaca	Pasteurized	3	170	154	161
		Raw	4	237	175	197
Milk (goat's)	Leche de cabra	Raw	5	320	220	276
Eggs:						
Hen's egg (white)	Huevo de gallina (clara)	Purchased at random in different markets	10	275	131	212
Hen's egg (yolk)[1]	Huevo de gallina (yema)	Purchased at random in different markets	10	1022	227	524
Turtle's egg (white)	Huevo de tortuga (clara)	Large sea turtle	3	39	30	34
Turtle's egg (yolk)	Huevo de tortuga (yema)	Large sea turtle	5	672	489	575
Fish:						
Dried salted codfish	Bacalao	Imported from Nova Scotia	6	49	30	39
Meat:						
Beefsteak	Bistec	Native animals	5	252	217	241
Goat's meat	Carne de cabro	Native animals	2	390	355	373
Pork loin	Carne de cerdo	Native animals	5	275	217	244
Miscellaneous:						
Casabe bread	Casabe	A crispy cracker made of meal prepared from cassava	3	26	18	22
Honey (bee's)	Miel de abeja	Local product	1	....	....	55
Molasses, edible	Melao	Prepared by concentrating cane sugar juice	3	80	51	61
Yeast (*Torula utilis*)	Levadura	Manufactured at the pilot plant of "Cía de Fomento de P. R."	1	....	....	5880

[1] High variation observed from sample to sample.

are not altogether uncommon among the poorer group of the population, the ones that usually consume a more restricted diet.

MATERIAL AND METHODS

Practically all the materials tested were purchased at various local markets in San Juan, Puerto Rico, and are representative of what the consumer buys.

Riboflavin was estimated by the microbiological procedure of Snell and Strong (1939). In those cases where decreasing riboflavin values or unreasonably high results were observed as the level of the sample was increased in the assay, the modification introduced to the original procedure by Strong and Carpenter (1942) was always followed. This modification consists of extracting the hydrolyzed sample with ether for the purpose of removing interfering free fatty acids.

Each sample assay was tested at four different levels (using duplicate tubes at each level). Those results falling outside of the range 0.05 to 0.25 microgram of riboflavin per tube were discarded. If the maximum variation of the remaining values, when calculated to the bases of micrograms of riboflavin per 100 grams of sample, were not over 10 per cent, all values were averaged to give the final result for that particular sample.

In the majority of cases five different samples of each material were assayed; the number of samples of each material assayed as well as the maximum and minimum values obtained and the average riboflavin content for the particular material are recorded (Table 1).

The vegetable materials were scientifically named according to Britton and Wilson (1932). The English common names used were those recorded by Britton and Wilson (1932) or by Otero and Toro (1931).

SUMMARY

The riboflavin content of 93 articles of food important in the diet of Puerto Rico and neighboring islands was determined by microbiological procedure.

ACKNOWLEDGMENT

The author wishes to express his appreciation to Professor F. M. Strong of the University of Wisconsin for his valuable suggestions in regard to the use of the microbiological procedure.

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Yeast (*Torula utilis*)..... Levadura | Manufactured at the pilot plant of "Cía de Fomento de P. R." | 1 | .... | .... | 5880

[1] High variation observed from sample to sample.

STUDIES ON VITAMIN CONTENT OF CANNED PINEAPPLE

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(Received for publication, April 13, 1945)

The survey reported here covered various types of pineapple, each type representing a different company or a variation in the processing technique or harvesting season. Vitamins A, B_1, B_2, C, pantothenic acid, and p-aminobenzoic acid were quantitatively determined. Bio-assays have been compared with chemical and physicochemical determinations. Where a discrepancy occurred between the methods, techniques were worked out to adapt the procedures to the specific conditions in pineapple.[2]

MATERIAL AND METHODS

VITAMIN A

Bio-Assay: Five types of commercial canned pineapple, here called (a), (b), (c), (d), and (e), were tested by the official—U. S. Pharmacopoeia (1939)—therapeutic method of assay with rats. Fresh cans were used for each feeding. The diet for the stock rats was as recommended by Coward (1939). In the test diet one-half of the starch was replaced by sugar to improve the texture and digestibility of the diet. One-half milligram of choline hydrochloride per gram of food was added to prevent haematuria.

Physicochemical Assay: A new method—Haagen-Smit, Jeffreys, and Kirchner (1943)—well suited to canned pineapple, for the isolation and purification of carotene involves the hydrolysis of xanthophylls with alcoholic potassium hydroxide and subsequent removal of the xanthophylls by means of phosphoric acid. The optical measurements were made with a Beckman photoelectric spectrophotometer and the concentration calculated by use of the specific absorption coefficient for β-carotene at 450 millimicrons.

VITAMIN B_1 (THIAMIN)

The "increase in weight" method of the Food and Drug Administration of the U. S. Department of Agriculture—Kline, Hall, and Morgan (1941)—was used for bio-assay. The groups of depleted rats were assembled within 30 days.

A Klett fluorometer was used in conjunction with the thiochrome method of Hennessey and Cerecedo (1939) for the chemical assay.

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[1] The authors wish to acknowledge with thanks the financial support of the Pineapple Research Institute of Hawaii for this program.

[2] The collaboration of R. Hassler and E. Woodbury on chemical assays is gratefully acknowledged.

VITAMIN B_2 (RIBOFLAVIN)

The microbiological method of Snell and Strong (1939) was adapted to the requirements of the material for bio-assay. (No chemical assay was made for riboflavin.)

VITAMIN C

The guinea-pig method of Zilva and Wells (1919), as modified by Coward and Kassner (1936) was used for bio-assay.

The 2,6 dichlorophenolindophenol method of Bessey (1939) was used for the chemical assay.

PANTOTHENIC ACID

The microbiological method of Pennington, Snell, and Williams (1940) was used for bio-assay. The vitamin was made available to the bacteria by enzymatic digestion. (No chemical test was made.)

p-AMINOBENZOIC ACID

The microbiological method of Lewis (1942) was used for bio-assay. The vitamin was made available to the bacteria by acid hydrolysis.

EXPERIMENTAL PROCEDURE

VITAMIN A

The bio-assay failed to show any evidence of vitamin A in the syrup; a summary of the vitamin A in International units per 100 grams of solid pineapple in five types of pineapple canned in the summer and in five types packed in the winter is given (Table 1).

TABLE 1

Vitamin A Content of Canned Pineapple

Summer pack			Winter pack	
Type	Biological assay	Physicochemical assay	Type	Physicochemical assay
	I.U./100 gm.			
a	145	135	f	58
b	125	106	g	79
c	143	129	h	93
d	138	134	i	63
e	147	135	j	55
Mean	139.6	127.8	Mean	69.6

Comments: 1. The biological assays give a consistently and significantly greater value (nine per cent) than the physicochemical method.

2. The amount of the vitamin in the winter pack averages 50 per cent of the amount in the summer pack.

VITAMIN B_1

The values in International units per 100 grams obtained by biological assay and chemical assay for five types of the winter crop are shown (Table 2).

TABLE 2

Vitamin B_1 Content of Canned Pineapple

Summer pack				Winter pack		
Biological assay		Chemical assay		Chemical assay		
Type	Aliquot parts of solid and syrup	Solid	Syrup	Type	Solid	Syrup
	I.U./ 100 gm.	*I.U./ 100 gm.*	*I.U./ 100 gm.*		*I.U./ 100 gm.*	*I.U./ 100 gm.*
a	29	28.7	29.3	f	21.3	22.3
b	....	28.7	29.0	g	21.9	24.1
c	31	30.0	29.7	h	25.0	27.1
d	27	28.7	29.7	i	23.6	23.3
e	....	29.7	30.0	j	22.9	24.9
Average	29	29.3	29.7	Average	22.9	24.3

Comments: 1. The biological and chemical assays are in close agreement.
2. The winter pack has slightly less thiamin than the summer pack.
3. The vitamin is equally distributed between solid and syrup.

Two shipments of fresh pineapple grown under different cultural conditions, were tested by chemical assay; they contained 34.7 and 35.3 I. U. per 100 grams. A check on the distribution of vitamin B_1 in fresh pineapple gave values as follows: center, 34.7; top, 37.3; bottom, 42.0; core, 36.0; near shell, 25.3; near core, 33.3 I. U. per 100 grams.

VITAMIN B_2

A summary is given of the riboflavin in the solid and the syrup of summer and winter pack of canned pineapple (Table 3).

TABLE 3

Vitamin B_2 Content of Canned Pineapple

Summer pack					Winter pack				
Solid			Syrup		Solid			Syrup	
Type	γ/100 gm.	Sherman units/ 100 gm.	γ/100 gm.	Sherman units/ 100 gm.	Type	γ/100 gm.	Sherman units/ 100 gm.	γ/100 gm.	Sherman units/ 100 gm.
(a) IS-OH J_2	28	11	16	6	f	33	13	23	10
(a) QHLS	44	18	26	10	g	48	19	32	13
b	37	15	13	5	h	33	13	31	12
c	31	12	10	4	i	34	14	26	10
d	40	16	31	12	j	45	18	24	10
e	37	15	18	7		....	....	....	....

Comments: 1. The distribution of the vitamin between solid and syrup is very inconsistent, perhaps owing to the relative insolubility of protein-linked riboflavin.
2. There is no significant difference between the summer and winter pack.

VITAMIN C

A summary is given of the amounts of vitamin C in International units per 100 grams found by the biological (guinea-pig) test and the chemical test in the summer pack, and by the chemical method in five types of winter pack of canned pineapple (Table 4). The values in brackets for Types c and d in the biological tests are those obtained in a repeat series at high levels of sample.

TABLE 4

Vitamin C in Canned Pineapple

Summer pack				Winter pack		
Biological		Chemical		Chemical		
Type	Aliquot parts of solid and syrup	Solid	Syrup	Type	Solid	Syrup
	I. U./ 100 gm.	*I. U./ 100 gm.*	*I. U./ 100 gm.*		*I. U./ 100 gm.*	*I. U./ 100 gm.*
a	92	98	102	f	102	138
b	....	99	92	g	96	119
c	70(81)	96	82	h	117	165
d	51(36)	72	64	i	115	157
e	....	96	84	j	165	222
Mean	70.4	92.2	84.8	Mean	119	160.2

Comments: 1. Owing to the known difficulty of the guinea-pig test, values from the bio-assay are less uniform than those from the chemical determinations.
2. The ascorbic acid content of the winter pack is for the solid 30 per cent and for the syrup 60 per cent greater than that of the summer pack.

For fresh pineapple [3] the average content was found to be 100 I. U. per 100 grams. The distribution of vitamin C in fresh pineapple was found to be in I. U. per 100 grams: center, 70; top, 82; bottom, 142; core, 130; near shell, 88; near core, 72.

PANTOTHENIC ACID[4]

A summary is given of the pantothenic acid content of three types of summer pack and two types of winter pack of canned pineapple determined by microbiological assay (Table 5).

TABLE 5

Pantothenic Acid in Canned Pineapple

Pack	Type	Aqueous suspension of solid	Takadiastase digest of solid	Syrup
		γ/100 gm.	*γ/100 gm.*	*γ/100 gm.*
Summer	K	74.2	116.3	53.1
	L	90.6	129.1	62.7
	M	90.2	129.3	55.3
	Mean	85.0	124.9	57.0
Winter	N	110.3	163.5	114.5
	T	106.9	162.8	109.9
	Mean	108.6	163.2	112.2

Comments: 1. The winter pack has in the solid 30 per cent and in the syrup 96 per cent more pantothenic acid than the summer pack.
2. The vitamin must be made available to the bacteria by preliminary digestion with an amylolytic enzyme.

[3] The collaboration of S. Gottlieb in the work on the fresh pineapple is gratefully acknowledged.

[4] The collaboration of Ruth P. Bolton in the work on pantothenic acid is gratefully acknowledged.

A papain digest of solid gave no increased yield over the aqueous suspension of solid. A combined papain-takadiastase digest gave the same yield as takadiastase alone.

p-AMINOBENZOIC ACID

A summary is given of the amounts of this vitamin in micrograms per 100 grams of the solid portion of canned pineapple, determined by microbiological assay (Table 6).

TABLE 6

p-Aminobenzoic Acid Content of Canned Pineapple

Type	Acid hydrolyzed solid	Type	Acid hydrolyzed solid
	γ/100 gm.		*γ/100 gm.*
K	16.8	N	18.5
L	22.4	T	15.4
M	25.7		
Mean	21.6	Mean	17.0

Comments: 1. Drastic hydrolysis is necessary to render the bound vitamin available to the bacteria used for this assay.

2. Probably some of the vitamin is destroyed during the hydrolysis, though further study is necessary to determine how much of it is lost.

Digestion of the pineapple by papain, by takadiastase, and by a combination of both enzymes did not significantly increase the yield over that of the undigested suspension. The filtrate of the suspension did not give as high a yield as the unfiltered suspension.

NICOTINIC ACID

Nicotinic acid was found to be present in small amounts but was not quantitatively determined.

SUMMARY

Vitamins A, B_1, B_2, C, pantothenic acid, and p-aminobenzoic acid were quantitatively determined in various types of canned pineapple, prepared by various canning methods and during various seasons of the year. In most cases parallel determinations by bio-assay and by chemical or physicochemical methods were made and compared.

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INFLUENCE OF CHEMICAL CHARACTERISTICS OF SOIL ON PRODUCTION OF CAROTENE IN LEAVES OF RYE[1]

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A description of the chemical properties of the surface soils of a group of soil sites in the vicinity of Midland, Douglas County, Kansas, has been published by Wynd and Romig (1943). Wynd and Noggle (1945a, 1946a) pointed out that the total amount of replaceable bases in these soils was their most important chemical characteristic related to the growth of oats and rye in this vicinity. These authors later reported (1945b, 1946b) that the amount of nitrogen controlled to a great extent the percentage of protein in the leaves of these species. Another paper by Wynd and Noggle (1945c) presented evidence that the amount of carotene in the leaves of oats was closely related to the same factors that were related to the amount of protein.

The vitamin content of plant tissues varies between very wide limits, a fact which is usually ignored by dietitians and by those who prepare the tables of the vitamin content of foods which appear in many textbooks on human nutrition. It is, therefore, of the greatest importance to establish as accurately as possible the effects of environmental factors on the accumulation of vitamins by plants. Since the vitamins are so intricately related to cellular physiology, the relationship between nutritional conditions and the ability of plant tissues to accumulate them is of especial interest to plant physiologists.

Very little is known concerning the effect of soil characteristics on the carotene content of plants and therefore the authors believed that it was worth while to extend their previously reported observation on oats to include the effects of a different set of 10 soils, at a different season of the year, and on another species. The former observations were based on oats harvested during the autumn of 1940, while the present study is based on rye harvested in the spring of 1941. The soils were not those used for the previous study, but they were all in the vicinity of Midland, Douglas County, Kansas. The determinations of carotene were made on the same samples which furnished the data for the reports on the growth of rye (1946a) and on the protein content of the leaves (1946b). Two additional samples are also included in the present study. The material was harvested at the jointing stage and dried immediately in an Arnold hot-air dehydrator. The determination of carotene was essentially that of Buxton and Dornbrow (1938) except that methyl alcohol was substituted for ethyl alcohol, and the heptane portion was concentrated on a hot plate in flasks

[1] The expenses incurred in the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

with vertical condensers instead of by vacuum distillation under nitrogen. The papers by Buxton and Dombrow (1938) and Wynd and Noggle (1945c) should be consulted for the details of the procedure.

The data (Table 1) are expressed in terms of milligrams of carotene per 100 grams of oven-dry leaf tissue, and also as yields of carotene in pounds per acre. The M.S.C. file numbers refer to the original samples permanently on file in the Laboratory of Plant Physiology at Michigan State College. The chemical data for each of the soils may be identified in the tables published by Wynd and Romig (1943) by referring to corresponding M.S.C. file numbers. The point numbers identify in the figures the soils from which each of the data was obtained.

TABLE 1

Accumulation of Carotene in Leaves of Rye Harvested at Jointing Stage

M.S.C. file No.		Field No.	Date	Point No.	Dry matter per acre	Carotene	Carotene yield per acre
Soil	Rye						
					lb.	*mg./ 100 gm.*	*lb.*
286	332	9	Apr. 10, 1941	1	144	32.0	.0461
285	334	10	Apr. 11, 1941	2	221	29.0	.0641
288	335	20	Apr. 12, 1941	3	379	33.0	.1251
284	336	19B	Apr. 13, 1941	4	500	28.3	.1415
287	337	22	Apr. 14, 1941	5	298	25.8	.0769
291	338	11	Apr. 17, 1941	6	947	32.0	.3030
289	339	15A	Apr. 20, 1941	7	1430	37.8	.5405
290	340	15B	Apr. 20, 1941	8	1970	32.3	.6363
292	341	1C	Apr. 21, 1941	9	833	35.9	.2991
283	331	1B	Nov. 15, 1940	10	.....	42.1	.....

The soils included in the present study may be classified into three groups on the basis of their chemical characteristics described by Wynd and Romig (1943). These groups may be summarized as follows:

Total replaceable bases low (5.5-15.8 me.). Soils clayey.

pH = 7.0 or more. Base saturation more than 100 per cent.

Fields 9, 10, 20, 19B, 22

Points 1, 2, 3, 4, 5

Total replaceable bases high (17.0-34.9 me.)

Soils not calcareous. pH less than 7.0. Base saturation less than 100 per cent.

Fields 15A, 15B, 1C, 1B

Points 7, 8, 9, 10

Soils calcareous. pH more than 7.0. Base saturation greater than 100 per cent.

Field 11

Point 6

It will appear below in the discussion of the figures that these soils fall into only two major groups if their effect on the carotene content of rye leaves be taken as the standard of comparison. Points 1 to 6, inclusive, represent soils having a low content of organic matter, consequently also a low base-exchange capacity, and comparatively small amounts of nitrogen, while Points 7 to 10, inclusive, represent soils having a high content of organic matter, consequently a high base-exchange capacity and comparatively large amounts of nitrogen. It is essential that these outstanding characteristics be borne in mind during the study of the data presented in the figures.

DISCUSSION

The base-exchange capacity of the soil is related to the concentration of carotene in the leaves of immature rye (Fig. 1A). The dark-colored loams, represented by Points 7 to 10, inclusive, are obviously superior to the calcareous clay loams, represented by Points 1 to 6, inclusive. The amount of organic matter in these soils determines to a great extent their base-exchange capacity and also their nitrogen content, consequently the distributions of the points in Figs. 1A, 11A, and 12A are very similar. In a strict sense, it would be impossible to separate the individual effects of these three related soil factors, yet it seems reasonable to assume that the nitrogen of the soil is the effective component of the group in influencing the carotene concentration in the leaves. Figure 1B shows that the small differences in the base-exchange capacity of the poorer soils, represented by Points 1 to 5, inclusive, are not related to the total yields of carotene, but the greater differences in the base-exchange capacity of the better soils, represented by Points 6 to 9, inclusive, show that this factor is important. Part of the relationship to the yield of carotene is due to the fact that this characteristic of the soil also is related to the yield of dry matter.

The amount of replaceable bases is shown (Fig. 2A) to be also related to the concentration of carotene in the tissue. Since greater amounts of replaceable bases are retained in soils having the greater base-exchange capacity, which in turn depends primarily on nitrogenous organic matter in the soils studied, it is difficult to recognize the effect of the replaceable bases themselves. The total yield of carotene is also affected since both its concentration and the yield of dry matter are associated with the higher amounts of replaceable bases.

The degree of base saturation appears from a study of Fig. 3A to be definitely related to the concentration of carotene in rye leaves. The degree of base saturation is the ratio of the total amount of replaceable bases obtained by leaching the soil with ammonium acetate to the base-exchange capacity. Since the base-exchange capacity is due to nitrogenous organic matter, this ratio might be regarded indirectly as the ratio of the bases to the nitrogen. The higher concentrations of carotene are associated with the lower ratios, which suggests that the amount of nitrogen in the soil far outweighs the effect of the bases in affecting the carotene content of the leaves. The effect of these ratios on the total yield of carotene appears in Fig. 3B where Points 1 to 6, inclusive, represent poorer soils. It is apparent that in these soils the amount of replaceable bases affects the

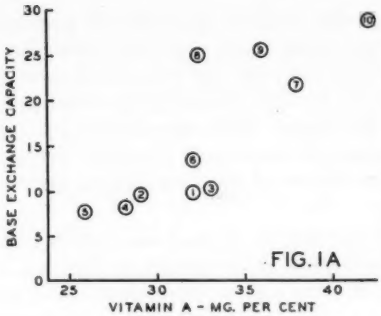


FIG. 1A

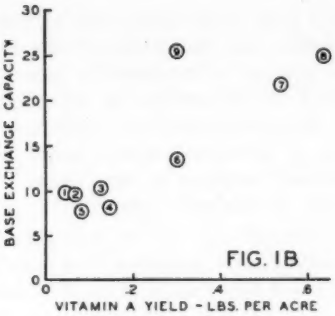


FIG. 1B

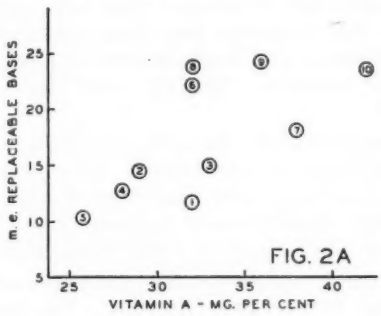


FIG. 2A

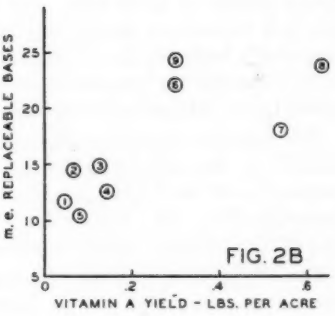


FIG. 2B

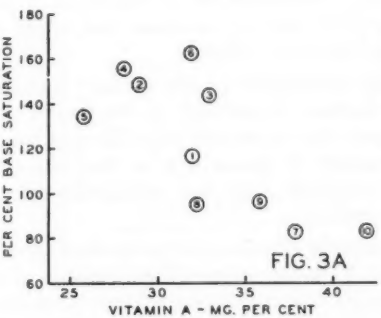


FIG. 3A

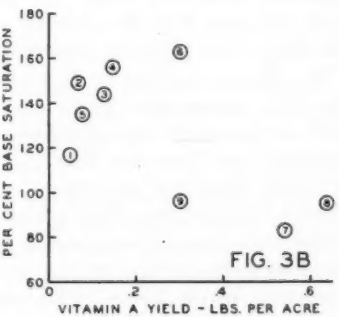


FIG. 3B

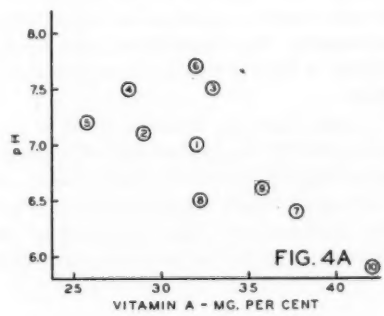


FIG. 4A

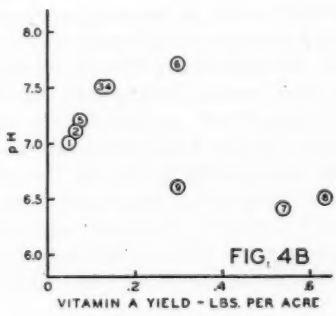


FIG. 4B

total yield more than does nitrogen. The authors pointed out previously (1946a) that the yield of dry matter from these soils was governed by the amount of replaceable bases rather than by the amount of nitrogen. The yield of carotene is governed more by the yields of dry matter than by the concentration of carotene in the tissue. Soils represented by Points 7 to 9, inclusive, contain large amounts of bases and nitrogen, hence the yield of carotene is very great. The degree of base saturation obviously is not intrinsically important.

The pH is a direct reflection of the degree of base saturation in this group of soils, and therefore the distribution of the points in Figs. 4A and 4B resembles that in Figs. 3A and 3B. The higher concentrations of carotene at the lower pH values are due to the higher amount of nitrogenous organic matter in these soils. The high yields of carotene from soils having lower pH values are due to the fact that these soils have large amounts of bases which increase the yield of dry matter and large amounts of nitrogen which increase the concentration of carotene. The pH itself does not appear to be intrinsically important.

The amount of replaceable calcium is shown (Fig. 5A) to bear a relationship to the concentration of carotene in the leaves. This positive relationship of the higher amounts of calcium to the total yield of carotene appears in Fig. 5B. This relationship is particularly apparent since the yield of dry matter is also related to the amount of replaceable calcium.

The effect of the ratios of replaceable calcium to the total replaceable bases on the concentration of carotene is presented (Fig. 6A). The lower ratios are associated with the higher concentration of carotene, a fact which indicates that the effect of calcium alone is less than that of all the total bases. The yields of carotene are similarly affected.

The greater amount of replaceable magnesium in the soils, as indicated (Fig. 7A), the greater is the concentration of carotene in the leaves. The effects on the total yield of carotene are also apparent (Fig. 7B). It should be pointed out, however, that the amount of magnesium in the soils studied closely parallels the amount of nitrogen, and this situation probably explains the apparent effect of magnesium.

The high ratios of replaceable magnesium to the total amount of replaceable bases in the soil are shown (Fig. 8A) to accompany high concentrations of carotene in the leaves. This suggests that the effect of the magnesium is greater than that of all the bases together. The favorable influence of the high ratios of magnesium to the total bases is due to the effect of the larger amounts of nitrogen which accompany the magnesium. An alternative, although less probable, explanation is that the ratio of magnesium to the total bases is intrinsically significant.

Points 1 to 6, inclusive (Fig. 8B), show that the smaller ratios of magnesium to the total bases are associated with higher yields of carotene. This relationship is due to the dominating effect of the total bases over that of magnesium alone and of the nitrogen which accompanies it. Points 7 to 9, inclusive, represent soils richer in bases and also in nitrogen; consequently the yield of dry matter and concentration of carotene are higher in the plants grown on these soils.

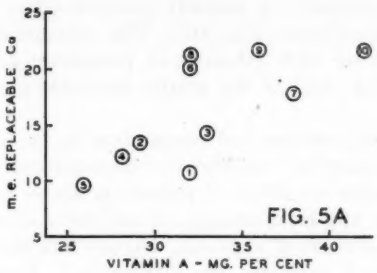


FIG. 5A

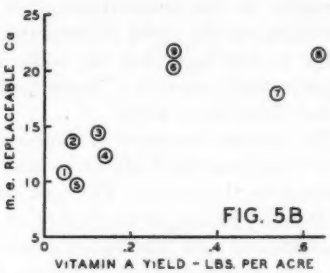


FIG. 5B

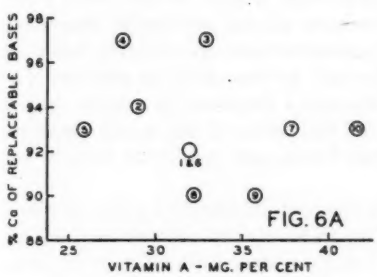


FIG. 6A

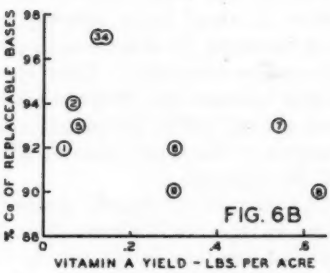


FIG. 6B

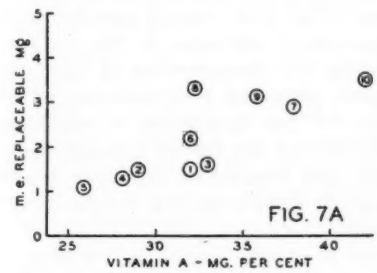


FIG. 7A

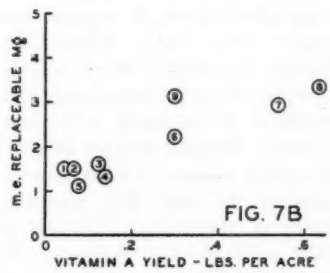


FIG. 7B

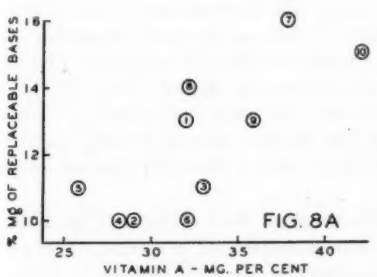


FIG. 8A

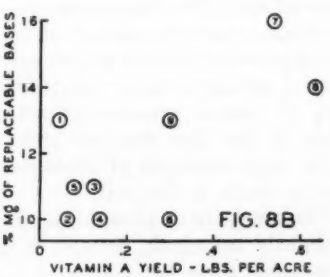


FIG. 8B

The amounts of replaceable potassium are shown (Fig. 9A) to be unrelated to the concentration of carotene. A vaguely positive effect of potassium on the yield of carotene is evident (Fig. 9B). This relationship is due to the fact that the soils having high amounts of potassium have a higher total amount of bases and the yield of dry matter is consequently greater from these soils.

The higher amounts of replaceable calcium and magnesium in the soil have been described above as accompanying the higher concentrations of carotene in the leaves. The lack of such an effect of potassium shows that the smaller ratios of this nutrient to the total amount of all bases should be associated with the higher concentrations of carotene. Figure 10A shows this relationship to exist if Points 4 to 6, inclusive, are disregarded. Figure 10B shows that some relationship exists with respect to the total yield of carotene if these three possibly erroneous points are again disregarded. Minor errors in the determination of potassium are unavoidable when small amounts are concerned. These errors may be too small to obscure a relationship between the absolute amounts and a response by plants, but such errors greatly affect the magnitudes of the ratios of the small amounts of potassium to the large amounts of total bases, and occasional erratic values must be expected.

The amount of organic matter in the soil indicated by the percentage of loss on ignition is shown (Fig. 11A) to be closely related to the concentration of carotene in rye leaves. It has been stated above that the organic matter in the soils included in the present study is nitrogenous and hence the apparent effect of organic matter (Fig. 11A) would parallel that of nitrogen (Fig. 12A). Since the amount of nitrogen in the soil is an especially important factor in governing the concentration of carotene in the leaves, the determinations of organic matter in these soils are of great importance in selecting soils suitable for the production of carotene in rye leaves. Organic matter affects the yield of dry matter not only because the organic matter is nitrogenous but also because of the bases which it retains in the soil. The relationship of organic matter to the yield of carotene is, therefore, especially great.

The amount of nitrogen in the soil is shown (Fig. 12A) to be a very important soil factor concerned with the concentration of carotene in the leaves of rye. The discussions above have called attention to the evidences which show that the amount of nitrogen in the soil is more important than the replaceable bases in governing the concentration of carotene. The great effect of nitrogen on the total yield of carotene is shown (Fig. 12B). The effect of higher amounts of nitrogen on the yield of carotene depends partly on the fact that the yield of dry matter also is greatly increased by the high amounts of replaceable bases which the nitrogenous organic colloids retain in the soil.

The amounts of phosphorus soluble in weak acid appear (Fig. 13A) to be slightly negatively related to the concentration of carotene. The yields of carotene from the poorer soils, represented by Points 1 to 5, inclusive (Fig. 13B), are greater from soils containing the most phosphorus. The same general relationship appears also for the richer soils represented by Points 6 to 8, inclusive. Point 9 seems to represent an erratic or erroneous

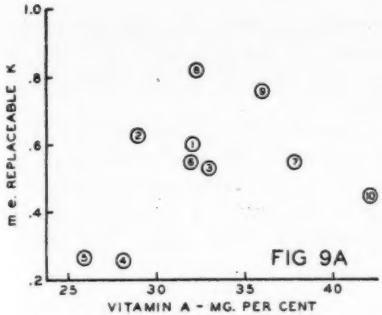


FIG 9A

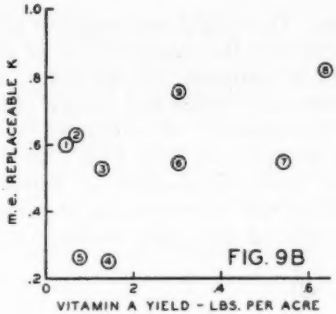


FIG. 9B

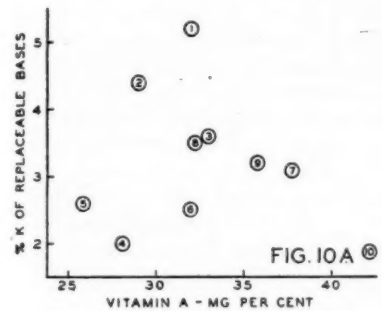


FIG. 10A

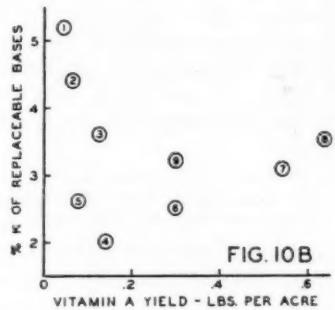


FIG. 10B

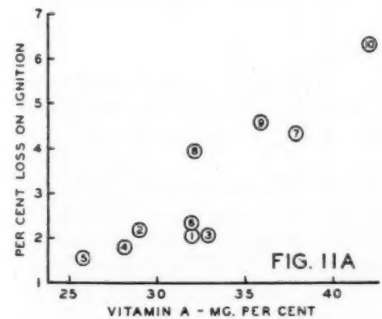


FIG. 11A

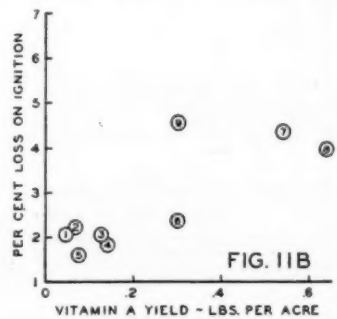


FIG. 11B

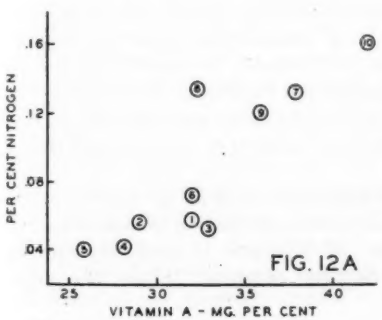


FIG. 12A

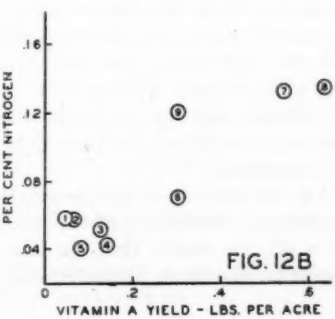
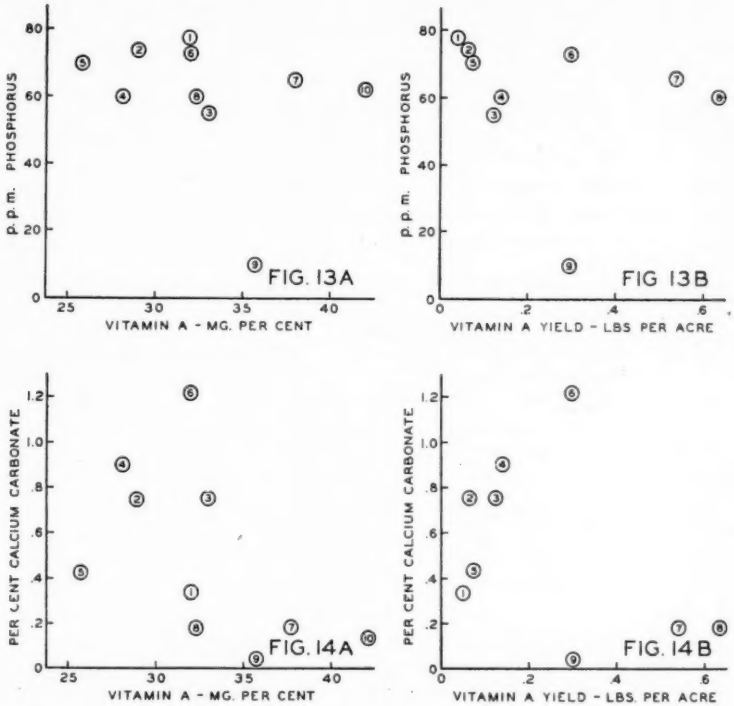


FIG. 12B

value. This slight and negative relationship between the amount of phosphorus and the concentration of carotene, on the one hand, and the total yield of carotene, on the other, were not evident in the study of oats reported by Wynd and Noggle (1945c).

The amounts of carbonate in the poorer soils, represented by Points 1 to 6, inclusive (Fig. 14A), are not related to the concentration of carotene. Soils represented by Points 7 to 10, inclusive, are noncalcareous, and the high concentration of carotene in the leaves of rye grown on them depends on the high content of nitrogen in these soils. The effect of


p. p. m. PHOSPHORUS
VITAMIN A - MG. PER CENT
FIG. 13A
p. p. m. PHOSPHORUS
VITAMIN A YIELD - LBS PER ACRE
FIG 13B
PER CENT CALCIUM CARBONATE
VITAMIN A - MG. PER CENT
FIG. 14A
PER CENT CALCIUM CARBONATE
VITAMIN A YIELD - LBS. PER ACRE
FIG. 14B

carbonate on the yield of carotene is apparent (Fig. 14B). The yields of dry matter from the poorer soils, represented by Points 1 to 6, inclusive, are closely dependent on the amounts of replaceable bases which accompany the carbonate, and therefore a great effect is exerted on the total yield of carotene. Points 7 to 9, inclusive, represent soils which have even higher amounts of bases together with large amounts of nitrogen. These soils therefore produce high yields of carotene, even though they are not calcareous.

An examination of all the relationships presented in the figures between the chemical properties of the soil and the concentration of carotene in the leaves of rye shows that the amount of nitrogen is probably the most important chemical characteristic affecting carotene. Since the organic

matter governs the base-exchange capacity and also the amount of nitrogen in the soil, the apparent effects of these three soil properties are the same, although the nitrogen is the significant component of the group.

The effect of soil properties on the total yield of carotene depends largely on their effect on the yield of dry matter, consequently the yield of carotene usually is governed by the total amount of replaceable bases in the soil. Replaceable calcium appears to be particularly important, while potassium appears to be without effect. Since the nitrogen governs the concentration of carotene and the replaceable bases govern the yield of dry matter, it is evident that the observed effect of either of these on the yield of carotene is greater than its specific effect.

Soils in the vicinity of Midland satisfactory for the production of carotene in the leaves of rye harvested at the jointing stage are characterized by chemical properties having at least the following magnitudes: base-exchange capacity, 15 milliequivalents per 100 grams of soil; total replaceable bases, 17 milliequivalents; replaceable calcium, 15 milliequivalents; replaceable magnesium, 2 milliequivalents; nitrogen, 0.08 per cent; and loss on ignition, 3.5 per cent.

The concentration of carotene, as well as its total yield, appears to be governed by the same soil factors that govern the percentage and yield of protein in the leaves.

The relationship of the various soil properties described above to the accumulation of carotene in rye leaves are of fundamental importance since they agree very closely with those obtained from a different species of cereal, grown during a different season of the year and on a different group of soils.

SUMMARY

The accumulation of carotene in the leaves of rye harvested at the jointing stage and grown on 10 soils in the vicinity of Midland, Douglas County, Kansas, is related to the chemical properties of the upper eight inches of soil.

The amount of nitrogen is the most important chemical property of the soil related to the concentration of carotene in the leaves.

The base-exchange capacity and also the percentage loss on ignition appear to be related to the concentration of carotene since they parallel the amount of nitrogen in the soil.

The higher amounts of replaceable calcium and magnesium also appear to be related to the concentration of carotene, although it is sometimes impossible to separate their individual effects from that of the nitrogenous organic matter which accompanies them.

The amount of replaceable potassium, and possibly also of weak-acid-soluble phosphorus, was not related to the accumulation of carotene in rye leaves.

The degree of base saturation, the pH values, and the amount of carbonate exhibit no specific relationship to the amount of carotene in the crop. They appear important only as they reflect or accompany the amount of replaceable bases or of nitrogen.

The yield of carotene was more closely dependent on the yield of dry matter than on the concentration of carotene in the leaves.

The concentration and yield of carotene appeared to be governed by the same soil factors as the percentage and yield of protein.

Soils in the vicinity of Midland satisfactory for the accumulation of carotene in the leaves of rye are characterized by soil properties having at least the following magnitudes: base-exchange capacity, 15 milliequivalents per 100 grams of soil; total replaceable bases, 17 milliequivalents; replaceable calcium, 15 milliequivalents; replaceable magnesium, 2 milliequivalents; nitrogen, 0.08 per cent; and loss on ignition, 3.5 per cent.

Soils containing less than 15 milliequivalents of replaceable bases, less than 0.08 per cent, and showing less than three per cent loss on ignition are unsatisfactory for the production of carotene in rye leaves.

The relationships described between the soil properties and the accumulation of carotene in the leaves of rye are of fundamental importance since they agree closely with those observed from a different species of cereal, grown during a different season of the year and on a different group of soils.

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VITAMIN P CONTENT OF VEGETABLES AS INFLUENCED BY CHEMICAL TREATMENT

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(Received for publication, April 19, 1945)

In previous papers from this laboratory the vitamin P content of some Chinese fruits and vegetables was described by Lo (1942) and Lo and Chang (1942). The present authors have also given a preliminary report of an investigation made in 1942 dealing with the promoting effect of certain chemical substances on vitamin P formation in vegetables in field experiments, Lo and Chen (1945). During 1943-44, this work was repeated and greatly extended and a much more complete study was made, the results of which are given here.

EXPERIMENTAL PROCEDURE

A series of field experiments was conducted to test the effect of some specific chemical substances (sulphates and nitrates of nickel, aluminum, zinc, magnesium, copper and iron, uranyl acetate, and molybdenum oxide) and a "complete fertilizer," singly and in combination. Chinese pepper (*Capsicum annuum*), pea (*Pisum sativum*, L.), and Chinese lettuce (*Lactuca Scariola* L. var.) were used as the experimental plants. The field experiment consisted of three blocks of plots laid out in randomized fashion and given 10 different treatments, each repeated three times. All plots were 3.5 by 5 feet with two rows of plants per plot. Each plot had six individual test plants. The outside rows in each plot served as guard rows, preventing contamination of any one plot by the chemical substances and fertilizer used on adjacent plots. Application of the complete fertilizer (ammonium sulphate: bone-meal: straw-ash, 3:1:4 by weight) was made at the rate of 488 pounds per acre before the seedlings were planted. All the specific chemical substances were used in a concentration of 0.0001 N, and 50 c.c. of solution was applied to each plant at 10-day intervals throughout the whole experimental period.

Estimation of vitamin P was carried out according to the method of Lorenz and Arnold (1941), and six determinations were made in all instances; results are shown (Tables 1 to 5).

DISCUSSION

From an inspection of the tables it is evident that there was a considerable variation in the vitamin P content of Chinese red peppers, pea shoots, green peas, Chinese lettuce leaves, and lettuce stems treated with various compounds. The extent of this variation differed with the compound used. The compounds of nickel, zinc, and molybdenum caused an increase in vitamin P in all the vegetables studied; while those of iron, copper, and aluminum did not seem to show a markedly favorable effect as compared with the controls, Lo and Wu (1943). This may well be due

TABLE 1

Effect of Some Chemical Substances on Vitamin P Content of Chinese Red Peppers (on Fresh Basis)

Treatment	Vitamin P content	Relative value
	mg./gm.	
O	1.450	100.00±3.29
CF[1]	1.435	98.96±2.51
$Fe_2(SO_4)_3$	1.481	102.13±3.32
$Fe_2(SO_4)_3$+CF	1.415	97.31±4.14
$Cu(NO_3)_2$	1.379	95.10±2.73
$Cu(NO_3)_2$+CF	1.360	93.79±2.37
$Ni(NO_3)_2$	1.755	121.34±3.75
$Ni(NO_3)_2$+CF	1.763	121.58±4.51
MoO_3	1.743	120.21±5.52
MoO_3+CF	1.715	118.27±4.93

[1] CF = Complete fertilizer (N–P–K).

TABLE 2

Effect of Some Chemical Substances on Vitamin P Content of Pea Shoots (on Fresh Basis)

Treatment	Vitamin P content	Relative value
	mg./gm.	
O	1.350	100.00±3.37
CF	1.335	98.88±4.14
$ZnSO_4$	1.603	118.74±2.53
$ZnSO_4$+CF	1.581	117.11±3.43
$Al_2(SO_4)_3$	1.411	104.51±1.37
$Al_2(SO_4)_3$+CF	1.353	100.22±4.11
$NiSO_4$	1.647	122.00±2.67
$NiSo_4$+CF	1.630	120.74±3.31
MoO_3	1.611	119.33±3.77
MoO_3+CF	1.671	123.77±2.41

TABLE 3

Effect of Some Chemical Substances on Vitamin P Content of Green Peas (on Fresh Basis)

Treatment	Vitamin P content	Relative value
	mg./gm.	
O	2.450	100.00±3.83
CF	2.471	100.89±4.35
$ZnSO_4$	2.913	118.89±5.31
$ZnSO_4$+CF	2.875	117.34±4.83
$Al_2(SO_4)_3$	2.250	91.83±5.59
$Al_2(SO_4)_3$+CF	2.341	95.55±6.33
$NiSO_4$	2.790	114.28±5.01
$NiSo_4$+CF	2.878	117.46±6.33
MoO_3	2.881	117.59±3.37
MoO_3+CF	2.922	119.26±3.17

TABLE 4

Effect of Some Chemical Substances on Vitamin P Content of Chinese Lettuce Leaves (on Fresh Basis)

Treatment	Vitamin P content	Relative value
	mg./gm.	
O	4.050	100.00±6.92
CF	3.872	95.60±7.92
$ZnSO_4$	5.570	137.53±3.91
$ZnSO_4$+CF	5.711	140.99±8.06
$NiSO_4$	5.312	131.11±6.13
$NiSO_4$+CF	5.430	134.07±9.15
$MgSO_4$	3.531	87.16±6.46
$MgSO_4$+CF	3.933	97.11±6.15
$UO_2(Ac)_2$	4.111	101.51±5.13
$UO_2(Ac)_2$+CF	4.131	102.00±4.78

TABLE 5

Effect of Some Chemical Substances on Vitamin P Content of Chinese Lettuce Stems (on Fresh Basis)

Treatment	Vitamin P content	Relative value
	mg./gm.	
O	.367	100.00±5.76
CF	.352	96.02±8.65
$ZnSO_4$	.565	154.16±5.89
$ZnSO_4$+CF	.526	143.58±3.44
$NiSO_4$	.514	140.09±3.68
$NiSO_4$+CF	.458	124.98±7.62
$MgSO_4$	.355	96.73±4.33
$MgSO_4$+CF	.359	97.82±8.11
$UO_2(Ac)_2$	.371	101.09±5.43
$UO_2(Ac)_2$+CF	.358	97.54±7.18

to the abundant occurrence of the compounds of Fe, Cu, and Al in the soil, so that probably no effect on the vitamin P content would be expected. The promoting effect of Ni, Zn, and Mo occurred consistently in various parts of the plants—sprouts, stems, leaves, and fruits.

The results obtained from the field experiments substantiate and support our previous conclusion in germination experiments with mung-beans, Lo and Wu (1943), that compounds of nickel, molybdenum, and zinc have the physiological function of promoting vitamin P formation in plant metabolism.

The effect of the complete fertilizer (providing nitrogen, phosphorus, and potassium) was not consistent and significant, confirming previous observations of Lo and Chen (1943).

SUMMARY

The effects of some specific chemical substances (sulphates and nitrates of nickel, aluminum, zinc, magnesium, copper and iron, uranyl acetate, and molybdenum oxide) and a "complete fertilizer," singly and in combination, upon vitamin P formation in vegetables were studied by field experiments.

The results obtained led to the conclusion that the compounds of nickel, zinc, and molybdenum have a physiological function of promoting vitamin P formation in plant metabolism.

The effect of the complete fertilizer was not consistent and significant.

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ASCORBIC ACID CONTENT OF CABBAGE SALADS

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(Received for publication, April 20, 1945)

A review of scientific literature revealed only a few investigations on the chemical determination of ascorbic acid content of raw cabbage salads. In one of these investigations Clayton and Borden (1943) studied the utilization of ascorbic acid in raw cabbage by feeding cabbage immediately after cutting it from the head. They found that the ascorbic acid of raw cabbage was utilized as well as, or possibly better than, that in the pure vitamin C tablets.

In a previous paper Clayton (1940) reported that salad dressings should not be allowed to come in contact with the food for any length of time before it is eaten if the maximum ascorbic acid value is to be obtained from the food. This study showed a higher loss of ascorbic acid from raw cabbage which had been combined with a mayonnaise made with cider vinegar than from the control which was combined with a mayonnaise made with distilled water. This same study also showed a slightly lower loss of ascorbic acid when lemon juice was used in place of the cider vinegar, namely, 57.1 to 57.9 per cent as compared with 67.8 to 69.2 per cent.

The method of preparing the raw cabbage for salads may affect the ascorbic acid content of the salad. Pyke (1942) found that the use of a household grater produced a greater destruction of the plant's cells than either the household shredder or a knife. The knife produced no destruction of ascorbic acid while the shredder gave a 10-per cent loss.

The purpose of the present study is to determine the ascorbic acid content of raw cabbage salads with and without salad dressings and held at room and at refrigerator temperatures.

EXPERIMENTAL PROCEDURE

The Flat Dutch cabbage used in this study was purchased at a retail store in Denton, Texas, during December, 1943; it was grown on sandy loam soil in South Texas and shipped to market by truck without refrigeration.

A representative sample of each raw cabbage head was obtained by the method of Lampitt, Baker, and Parkinson (1943). The head was trimmed as for cooking, then held with the central stalk in a vertical position and quartered by two vertical cuts, the cuts separating the stem longitudinally into four approximately equal parts, each attached to a quarter of the cabbage. Wedges directly opposite to one another were cut, from one pair of opposite quarters, each wedge containing stalk, inner leaves, and outer leaves in approximately the same proportions as did the whole cabbage

[1]Presented in partial fulfillment of requirements for the master's degree.

head. The wedges were cut into small pieces with a sharp, stainless-steel knife, the cuts being made as nearly as possible at right angles to the leaves, which were thereby reduced to pieces approximately one-fourth inch square. These small pieces of cabbage were mixed together and a representative sample weighing from 20 to 25 grams was taken for an ascorbic acid analysis.

The ascorbic acid values of samples from each wedge of six heads of cabbage (Table 1) indicate that the maximum difference in ascorbic acid content of the four wedges from any one head of cabbage was 1.8 milligrams per 100 grams, with a minimum of 0.9. From these data it is assumed that any increment or decrement in excess of these amounts of ascorbic acid of the raw cabbage or the raw cabbage salad is not due to discrepancies in sampling.

TABLE 1

Ascorbic Acid Content of Wedges Used in Sampling of Six Fresh Heads of Cabbage

Heads of cabbage tested	Ascorbic acid content of each wedge				Ascorbic acid average	Maximum difference
	1	3	2	4		
	mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	
1	28.8	27.9	29.7	29.2	28.9	1.8
2	33.4	32.0	33.7	32.5	32.9	1.7
3	26.3	26.3	27.4	26.8	26.7	1.1
4	32.1	31.5	31.5	30.4	31.4	1.7
5	30.1	29.2	30.1	29.3	29.7	0.9
6	26.2	26.6	26.2	27.5	26.6	1.3

In the home the cabbage is not eaten immediately after cutting from the head as described by Clayton and Borden (1943), nor is it possible to mix a salad and serve it at once. The ascorbic acid values obtained in previous studies do not then represent home conditions. As mentioned before, Pyke (1942) reported no loss of ascorbic acid when a knife was used in preparing the cabbage while a shredder gave a 10-per cent loss, but the latter is a much quicker method and gives a uniform product; consequently, a preliminary study was undertaken to determine the effect of allowing shredded cabbage to stand before testing. Four heads of cabbage were used in comparing the ascorbic acid content of cabbage prepared with a shredder and with a stainless-steel knife; each portion was allowed to stand 20 minutes before the second testing; results are given (Table 2). From this table it is evident that there is little difference in the ascorbic acid content prepared by shredding and by cutting with a knife after the cabbage has stood 20 minutes at room temperature.

To obtain family portions a technique similar to the one employed above was used to sever the heads into two approximately equal parts which were then rapidly shredded with a household vegetable cutter into each of two crockery bowls. To one bowl was added four teaspoons of apple-cider-vinegar mayonnaise; this was tossed to mix it well and then exposed to room temperature for zero minutes, 30 minutes, and for five hours. The other bowl of shredded cabbage was exposed to room temperature without vinegar mayonnaise for zero and 30 minutes. This process

was repeated with each of 10 cabbages. Another set of 10 heads of cabbage was treated in a like manner except that the bowls were covered with waxed paper and placed in an electric refrigerator for 30 minutes and for five hours.

Following this procedure, lemon-juice mayonnaise and apple-cider-vinegar French dressing were added to different portions of shredded cabbage and exposed for the same time intervals to room and to refrigerator temperatures.

TABLE 2

Ascorbic Acid Content of Cabbage Salads Using a Shredder and a Sharp Knife

Tool used	Time after shredding	Cabbage head	Ascorbic acid	Loss
	min.		*mg./100 gm.*	*pct.*
Knife	0	No. 1	32.2	.....
Knife	20	No. 1	21.1	34.5
Shredder	20	No. 1	20.3	37.8
Knife	0	No. 2	29.7	.....
Knife	20	No. 2	19.1	35.7
Shredder	20	No. 2	18.5	37.0
Knife	0	No. 3	28.7	.....
Knife	20	No. 3	18.3	36.0
Shredder	20	No. 3	18.1	35.9
Knife	0	No. 4	29.9	.....
Knife	20	No. 4	17.5	41.5
Shredder	20	No. 4	17.3	42.1

The vinegar mayonnaise, which was prepared with apple-cider vinegar every fifth day, and the lemon-juice mayonnaise, which was prepared daily, were kept in an electric refrigerator in closed containers. The French dressing which was made with apple-cider vinegar was prepared as needed and stored in the refrigerator also. The ascorbic acid content of these salad dressings was determined for each new batch. Neither the French dressing nor the mayonnaise made with apple-cider vinegar contained ascorbic acid. The average of the eight different batches of lemon-juice mayonnaise averaged 4.05 milligrams per 100 grams of dressing with a range of 3.70 to 4.17.

The ascorbic acid determinations obtained in this study used the modified chemical titration method of Burrell, Brown, and Ebright (1940) and the Waring blendor extraction method of Davis (1942). Instead of filtering the extract from the Waring blendor through a Büchner funnel it was centrifuged for 10 minutes.

An acid blank was run in obtaining the correction factor for the dye solution used in making the final calculations. Three 20- to 25-gram samples were taken of each salad and three aliquots of each sample were titrated. The mean and the average deviations of the ascorbic acid content of the salads from each group of 10 cabbages are given (Table 3).

DISCUSSION OF RESULTS

The milligrams of ascorbic acid per 100 grams of freshly shredded Flat Dutch cabbage (purchased at a local retail store) obtained in this

TABLE 3

Ascorbic Acid Content of Shredded Cabbage Salads With and Without Salad Dressing Held at Room and Refrigerator Temperatures

Number of heads used	Freshly cut—mean and av. dev.	Without salad dressing					
		In room		In refrigerator			
		30 min. mean and av. dev.	Retention mean and av. dev.	30 min. mean and av. dev.	Retention mean and av. dev.	5 hr. mean and av. dev.	Retention mean and av. dev.
	mg./100 gm.	*mg./100 gm.*	*pct.*	*mg./100 gm.*	*pct.*	*mg./100 gm.*	*pct.*
10	30.5±1.4	19.1±1.9	63±5.1	.....	.....	.....	.....
10	29.1±1.6	.....	.....	20.9±1.7	72±1.4	19.1±1.4	65±4.3
10	29.6±2.1	18.9±2.7	64±4.6	.....	.....	.....	.....
10	30.0±1.2	.....	.....	23.1±2.2	78±4.4	21.3±1.8	71±3.0
10	30.6±1.7	23.0±1.4	75±4.8	.....	.....	.....	.....
10	30.8±2.4	.....	.....	24.9±2.1	81±2.4	23.2±2.6	76±4.1
Mean and av. dev.	.....	.....	67±5	.....	77±3	.....	71±4

Number of heads used	Freshly cut—mean and av. dev.	Type of salad dressing used	With salad dressing							
			In room				In refrigerator			
			30 min. mean and av. dev.	Retention mean and av. dev.	5 hr. mean and av. dev.	Retention mean and av. dev.	30 min. mean and av. dev.	Retention mean and av. dev.	5 hr. mean and av. dev.	Retention mean and av. dev.
	mg./100 gm.		*mg./100 gm.*	*pct.*	*mg./100 gm.*	*pct.*	*mg./100 gm.*	*pct.*	*mg./100 gm.*	*pct.*
10	30.5±1.4	French dressing	22.9±1.6	75±3.3	21.7±1.2	71±3.7	.....	.....	.....	.....
10	29.1±1.6	French dressing	.....	.....	.....	.....	23.8±2.0	82±3.2	22.4±2.1	77±3.5
10	29.6±2.1	Vinegar mayonnaise	21.5±2.0	73±4.0	20.0±1.8	68±5.0	.....	.....	.....	.....
10	30.0±1.2	Vinegar mayonnaise	.....	.....	.....	.....	24.7±1.9	82±4.0	22.8±2.0	76±2.9
10	30.6±1.7	Lemon-juice mayonnaise	27.1±1.9	89±1.8	25.0±1.8	82±4.7	.....	.....	.....	.....
10	30.8±2.4	Lemon-juice mayonnaise	.....	.....	.....	.....	28.7±2.4	93±1.6	27.4±2.6	89±3.0
Mean and av. dev.	.....		.....	79±3	.....	74±5	.....	86±3	.....	81±3

study ranged from 26.6 to 32.9, with a mean of 29.9. These values are considerably lower than those reported for freshly harvested cabbage by other investigators. Thus, Van Duyne, Chase, and Simpson (1944), who used cabbage freshly harvested between October eighth and tenth, reported an initial ascorbic acid value ranging from 42 to 48. Cabbages which averaged 47 milligrams were obtained from their local market by Ireson and Eheart (1944) but the time of year and the variety of cabbage purchased are not given. Gould, Tressler, and King (1936), found an average of 29 milligrams per 100 grams for three fall types of cabbage tested.

In Pyke's study (1942), showing that the household shredder and a sharp knife produced less loss of ascorbic acid than the household grater, it was found that the sharp-knife method showed less loss on standing 20 minutes than the household shredder, namely 0 to 12 per cent, respectively. In the present study higher losses were obtained for both methods and less discrepancy between the two methods, 36.9 and 38.2 per cent, respectively (Table 2). The similarity of results in this case may be due to the fact that half of a small head of cabbage, from 200 to 250 grams, was exposed at a time to air at room temperature. As a result of the very small difference in loss, the household shredder was used in preparing the salads because it was faster.

In the preparation of "slaw" salads some time elapses between the shredding and the adding of the salad dressing. Van Duyne, Chase, and Simpson (1944) anticipated this in their study which had been completed but not published prior to the completion of the present study. The former study used approximately 100 grams of knife-shredded cabbage for exposure to air. Cabbages purchased on the local market gave greater losses than the freshly harvested; total loss due to advance preparation (one hour in air) did not exceed 6 $\pm$ 1 per cent. In the present study (Table 3) the ascorbic acid loss of 30 heads exposed to room temperature for 30 minutes was 33 $\pm$ 5 per cent. Whereas, the 30 heads exposed to refrigerator temperature lost 23 $\pm$ 3 and 29 $\pm$ 4 per cent during 30 minutes and five hours, respectively.

Clayton (1940) reported that salad dressings should not come in contact with food for any length of time before it is eaten if the maximum ascorbic value is to be obtained from the food. The data from the present study do not confirm this. The salad dressing served as a protective agent both at room and at refrigerator temperatures. The retention values for the former are 79 $\pm$ 3 and 74 $\pm$ 5 per cent and for the latter, 86 $\pm$ 3 and 81 $\pm$ 3 per cent for 30 minutes and for five hours, respectively. There is less difference in the retention values of French and vinegar mayonnaise salads at refrigerator temperature than at room temperature. However, in both instances the retention is slightly less than in the salads made with lemon-juice mayonnaise. This is not due to ascorbic acid contributed by the lemon-juice mayonnaise since that value would not exceed 0.2 milligram per 100 grams of cabbage, or approximately 0.7 per cent of the total ascorbic acid content.

Clayton (1940) also found less ascorbic acid loss when lemon juice was used in the place of cider vinegar in mayonnaise. Her losses for both

lemon-juice and cider-vinegar mayonnaise were greater than those shown in this study (Table 3).

The pH of the salad dressings was determined; the values were 3.06, 3.14, and 4.09 for the French dressing, lemon-juice mayonnaise, and vinegar mayonnaise, respectively. This suggests that the difference in retention of ascorbic acid is not primarily one of pH since the two with similar values, French dressing and lemon-juice mayonnaise, gave the minimum and maximum retentions. Both of the mayonnaise salad dressings served to protect the ascorbic acid of raw cabbage more than did the French dressing. This confirms Clayton's (1940) observation that mayonnaise made with distilled water in place of an acid gave a smaller loss of ascorbic acid than that made with vinegar. Consequently, the type of emulsion represented by mayonnaise is probably responsible for the greater retentions observed in the present study, while the lower pH of the lemon-juice mayonnaise further increased the retention.

SUMMARY

French dressing, vinegar mayonnaise, and lemon-juice mayonnaise all aid in the retention of ascorbic acid in raw, shredded cabbage salads.

Shredded cabbage mixed with salad dressing retains more ascorbic acid at room temperature than that without salad dressing.

The lemon-juice mayonnaise offers more protection at both room and refrigerator temperatures than either the French dressing or the vinegar mayonnaise.

Both the vinegar and lemon-juice mayonnaise gave higher ascorbic acid retentions than the French dressing.

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INFLUENCE OF CHEMICAL PROPERTIES OF SOIL ON PRODUCTION OF VITAMIN C IN LEAVES OF RYE[1]

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The chemical properties of a group of soils in the vicinity of Midland, Douglas County, Kansas, have been described by Wynd and Romig (1943). Wynd and Noggle have published a series of papers describing the effects of these properties on the growth of oats (1945a) and rye (1946a) harvested at the jointing stage, on the percentage and yield of protein in the leaves of oats (1945b) and rye (1946b), on the concentration and yield of carotene in the leaves of oats (1945c) and rye (1946c), and on the accumulation of vitamin C (ascorbic acid) in the leaves of oats (1945d). These authors found that the growth of these species was governed primarily by the total amount of replaceable bases in the soils. The concentration of protein and of carotene in the leaves of both rye and oats depended primarily on the amount of nitrogen in the soil. The total yields of these substances, in pounds per acre, were determined in both species by the yield of dry matter rather than by their percentages in the crop.

An important result of these studies was the discovery that the specific chemical properties of the soil influenced similarly the responses of both oats and rye, even though these species were grown during different seasons of the year, and on different groups of soils. Since the plants were influenced by a great variety of environmental factors, both known and unknown, it follows that specific factors which consistently exert observable effects under diverse conditions must be of fundamental importance. Since the magnitude of the observable effect of any factor is influenced by other factors in the environment, it would follow, also, that the persistent observable influence of a specific factor is of greater fundamental importance than is indicated by its observed numerical magnitude.

The effects of soil properties on the vitamin C content of oat leaves were reported by the authors (1945d) to be less easily recognized than their effects on growth, or on the accumulation of protein and carotene. The amount of vitamin C (ascorbic acid) in plant tissues is particularly variable, even when the plants are grown under apparently similar conditions. It is, therefore, of interest to learn if the relationships previously described between the chemical properties of the soil and the vitamin C content of oat leaves persist for the leaves of rye grown on a different group of soils and during a different season of the year.

The present paper reports the effects of certain chemical properties of eight soils in the vicinity of Midland, Douglas County, Kansas, on the

[1] The expenses incurred during the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

vitamin C content of rye leaves grown during the spring of 1941. The tissues studied were the same samples which furnished the previously published data on the yield (1946a), the contents of protein (1946b), and carotene (1946c). The plants were harvested at the jointing stage and quickly dried in an Arnold hot-air dehydrator. The determinations of vitamin C were made immediately by triturating one gram of the powdered sample with two or three grams of purified Ottawa sand in 25 milliliters of an extracting solution. The extracting solution contained two per cent of metaphosphoric acid and three per cent of trichloroacetic acid. One milliliter of the filtrate from the triturated sample was titrated with a standard solution of 2,3-dichlorophenolindophenol prepared by dissolving 1.5 grams of the sodium salt of the dye in one liter of dioxane which contained one per cent of acetic acid. The details of the procedure are described in the previous paper by Wynd and Noggle (1945d).

TABLE 1

Vitamin C (Ascorbic Acid) in Leaves of Rye

M.S.C. file No.		Field No.	Point No.	Date	Dry matter per acre	Vitamin C	Vitamin C yield per acre
Soil	Rye						
					lb.	*mg./ 100 gm.*	*lb.*
286	332	9	1	Apr. 10, 1941	144	372	0.54
285	334	10	2	Apr. 11, 1941	221	338	0.75
288	335	20	3	Apr. 12, 1941	379	387	1.47
284	336	19B	4	Apr. 13, 1941	500	291	1.46
287	337	22	5	Apr. 14, 1941	298	2	0.87
291	338	11	6	Apr. 17, 1941	947	226	2.14
289	339	15A	7	Apr. 20, 1941	1430	274	3.92
290	340	15B	8	Apr. 20, 1941	1970	274	5.40

The data presented (Table 1) are expressed as milligrams of vitamin C per 100 grams of oven-dry tissue and as yields in pounds per acre. The M.S.C. file numbers refer to the original samples permanently preserved in the Laboratory of Plant Physiology at Michigan State College. The chemical data for each of the soils may be identified in the tables published by Wynd and Romig (1943) by referring to corresponding M.S.C. file numbers. The point numbers identify in the figures the soils from which each of the data was obtained.

The soils included in the present study may be classified into three groups on the basis of the amounts of replaceable bases which they contain and their degree of base saturation. Soils of Fields 9, 10, 20, 19B, and 22 (Points 1 to 5, inclusive) are clayey and contain from 5.5 to 15.8 milliequivalents of replaceable bases per 100 grams. Their degree of base saturation is greater than 100 per cent and their pH values are 7.0 or more. Soils from Fields 11, 15A, and 15B (Points 6 to 8, inclusive) are dark-colored loams, containing from 17 to 34.9 milliequivalents of replaceable bases per 100 grams. The soil of Field 11 (Point 6) is calcareous and

is saturated with bases, while the soils from Fields 15A and 15B are not calcareous and are not saturated with bases. The recognition of these major differences greatly facilitates the interpretation of the data presented in the figures.

DISCUSSION

The base-exchange capacity of soils is not strikingly related to the concentration of vitamin C in the leaves of immature rye as indicated (Fig. 1A). A slight negative relationship can be detected between the magnitudes of the base-exchange capacity and the concentration of the vitamin. This effect is opposite to that previously observed in oats. Since the nitrogenous organic matter in these soils closely parallels their base-exchange capacities, the distribution of the points in Fig. 1A is almost identical with their distribution in Fig. 11A, which relates the percentage loss on ignition to the vitamin content, and in Fig. 12A, which relates the nitrogen content of the soil to the concentration of the vitamin C. The total yield of vitamin C is shown (Fig. 1B) to be dependent on the base-exchange capacity. This result depends on the relationship which the base-exchange capacity, and other chemical properties of the soil which are associated with it, bear to the yield of dry matter. This result is in complete agreement with that found with oats.

The total amount of replaceable bases is shown (Fig. 2A) to be inversely related to the vitamin C concentration in the leaves. This relationship is directly opposite to that previously observed for oats. The total yield of vitamin C, on the other hand, is dependent on the amount of replaceable bases. This result depends on the fact that the yield of dry matter is governed primarily by the amount of replaceable bases, and it agrees with the data obtained from oats.

The effect of the degree of base saturation is presented (Fig. 3A); Points 1 to 6, inclusive, represent soils saturated with bases and their distribution indicates that the lower degrees of base saturation are associated with the higher concentrations of vitamin C. Since the degree of base saturation in the soils studied may be regarded indirectly as the ratio of the total amount of replaceable bases to the nitrogenous organic matter, Wynd and Romig (1943), the effect of this ratio might be due to the fact that increasing amounts of nitrogen are more effective than the total amount of replaceable bases in controlling the concentration of vitamin C in the leaves. Points 7 and 8 are unsaturated soils, rich in nitrogen, and it appears (Fig. 3A) that these soils affect the vitamin C content differently than do soils saturated with bases. The yield of vitamin C is shown (Fig. 3b) to be closely related to the degree of base saturation in the group of saturated soils represented by Points 1 to 6, inclusive. This is due to the fact that larger amounts of replaceable bases are present in the more highly saturated soils, and these bases govern the yield of dry matter. Points 7 and 8, which represent soils unsaturated with bases, are located outside of the general group because the large amount of bases which they contain produce high yields of dry matter. The degree of base saturation is seen to have no intrinsic significance. These data agree with those obtained in the study of oats.

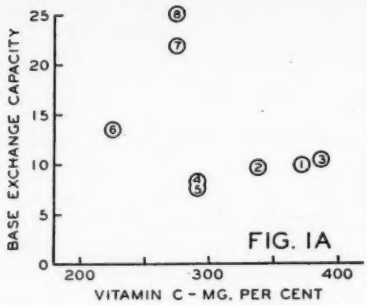


FIG. 1A

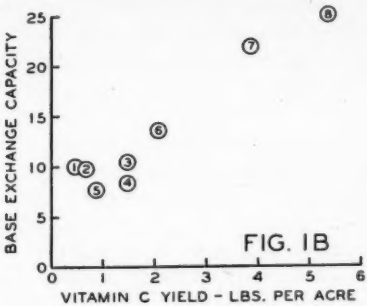


FIG. 1B

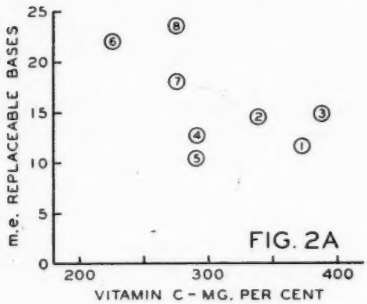


FIG. 2A

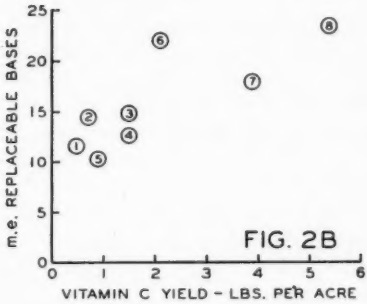


FIG. 2B

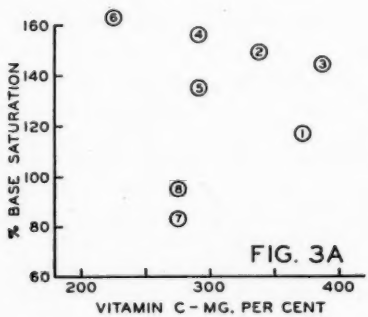


FIG. 3A

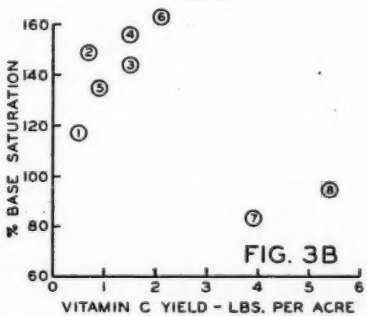


FIG. 3B

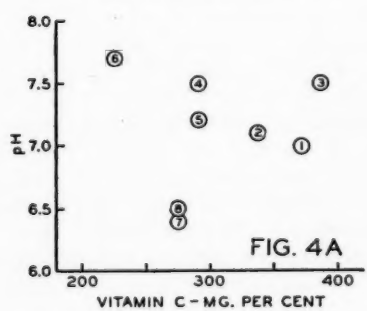


FIG. 4A

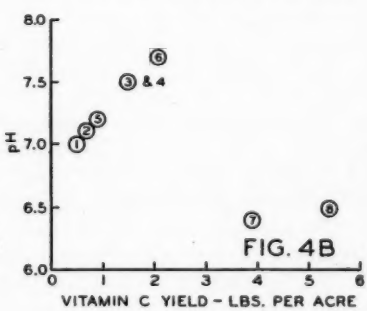


FIG. 4B

The pH of these soils closely reflects the degree of base saturation, hence the distribution of the points in Figs. 4A and 4B resembles that in Figs. 3A and 3B.

The amount of replaceable calcium is inversely related to the concentration of vitamin C in the leaves, a relationship which is contrary to that observed in oats (Fig. 5A). The total yield of vitamin C is shown (Fig. 5B) to be related to the amount of replaceable calcium in the soil. This fact depends on the favorable effect of replaceable calcium on the yield of dry matter, a result which agrees with that observed in oats.

The relationship between the replaceable calcium and the total replaceable bases to the concentration of vitamin C is shown (Fig. 6A) to be indefinite or vaguely positive. Its effect on the total yield of vitamin C, however, is definitely negative. This latter effect depends on the fact that the yield of dry matter is affected less by replaceable calcium than by the total amount of all the bases. This result agrees with that observed in oats.

The amounts of replaceable magnesium are inversely related to the concentration of vitamin C in the leaves (Fig. 7A). This inverse relationship of magnesium to the concentration of vitamin C in the leaves of rye is directly opposite to the previously observed relationship of magnesium to vitamin C in oat leaves. This relationship is similar to that of replaceable calcium. The amount of replaceable magnesium in these soils varies directly as does the amount of nitrogenous organic matter, and therefore distribution of the points in Fig. 7A is nearly identical with their distribution in Figs. 11A and 12A. The favorable effect of replaceable magnesium on the total yield of vitamin C depends on its effect on the yield of dry matter. This result agrees with the data obtained from oats.

The ratio of replaceable magnesium to the total amount of bases is shown (Fig. 8A) to bear no evident relationship to the concentration of vitamin C. The effect of magnesium, or perhaps the nitrogenous organic matter which accompanies it, appears to be about equal to the combined effect of all the replaceable bases. The effect of this ratio on yield of vitamin C is presented (Fig. 8B). In the soils saturated with bases (represented by Points 1 to 6, inclusive) the lower ratios are associated with the higher yields because the effect of magnesium, or the properties of the soil associated with it, on the yield of dry matter is less than the combined effect of all the bases. Points 7 and 8 represent high yields probably because the high amount of bases which these soils contain produced a markedly high yield of dry matter.

The amount of replaceable potassium bears no relation to the concentration or total yield of vitamin C.

The ratio of replaceable potassium to the total amount of replaceable bases is shown (Fig. 10A) to be directly related to the concentration of vitamin C. This fact suggests that the effect of potassium was greater than that of all the bases, including potassium. This apparently irrational explanation can be made to seem reasonable by assuming that some other favorable substance, or condition, accompanies potassium and thereby so augments its apparent effect that it appears to exert more influence than the combined effects of all bases. There is, however, no data to support this interpretation. The yields of vitamin C from the saturated soils (rep-

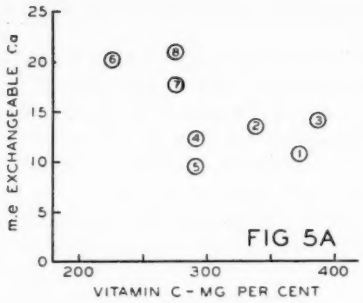


FIG 5A

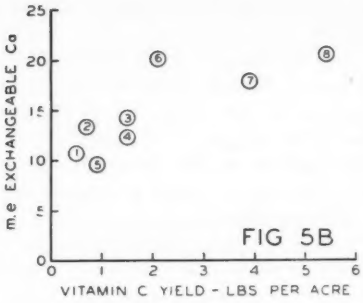


FIG 5B

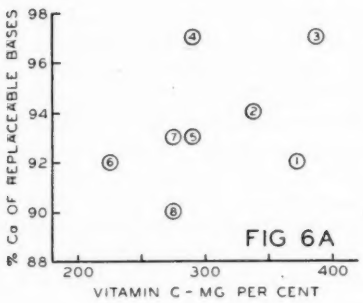


FIG 6A

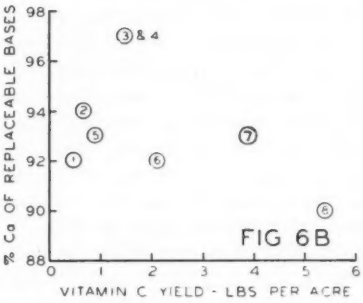


FIG 6B

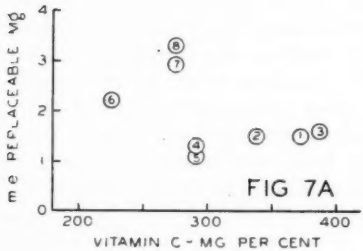


FIG 7A

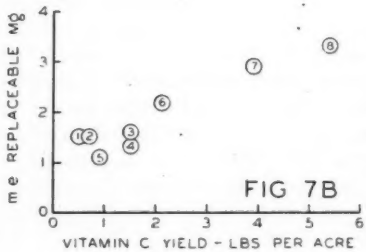


FIG 7B

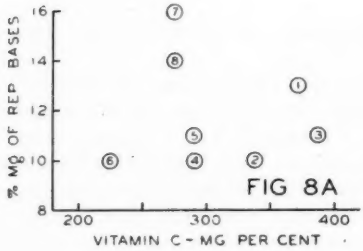


FIG 8A

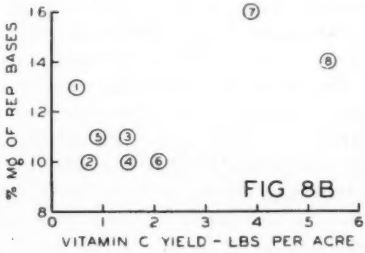


FIG 8B

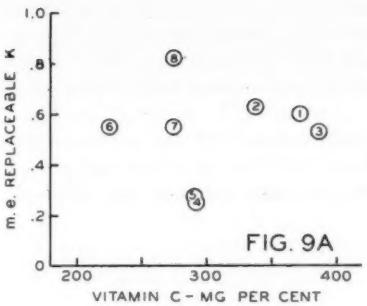


FIG. 9A

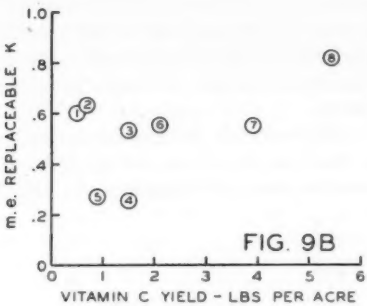


FIG. 9B

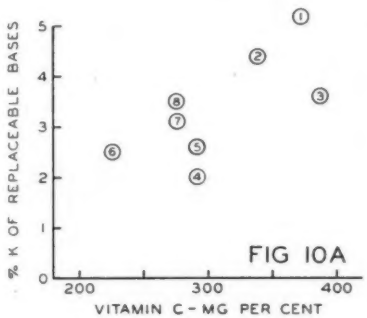


FIG 10A

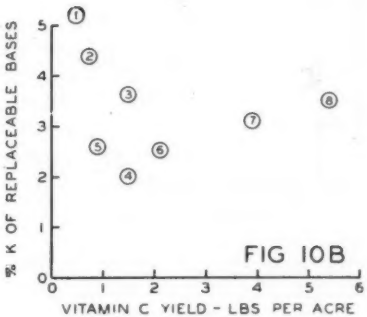


FIG 10B

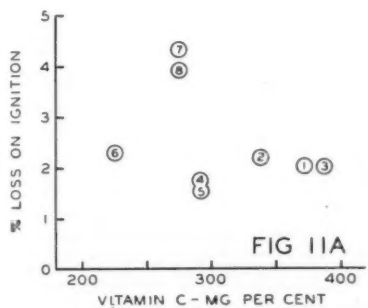


FIG 11A

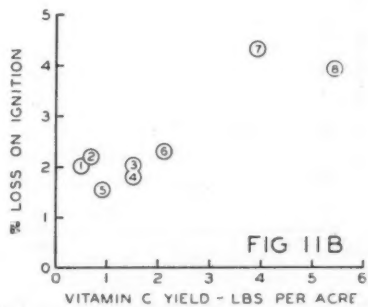


FIG 11B

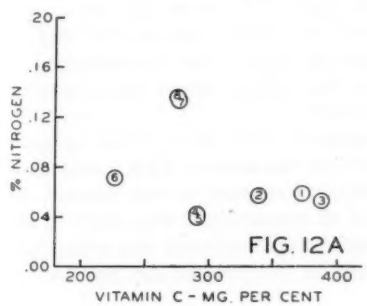


FIG. 12A

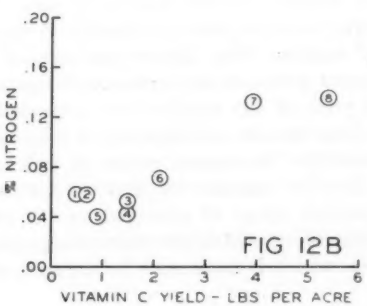
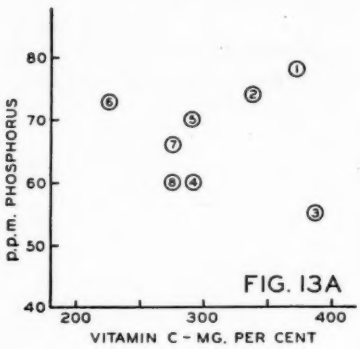
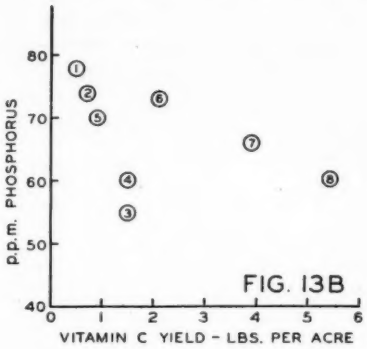


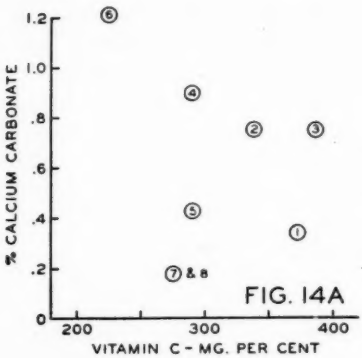
FIG 12B

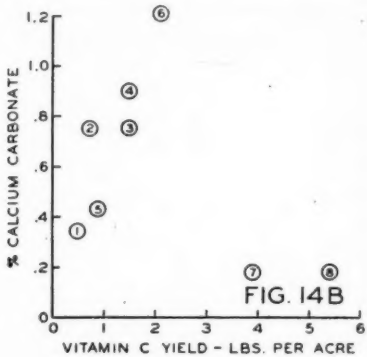
resented by Points 1 to 6, inclusive) are shown (Fig. 10B) to increase as the ratio of potassium to the total replaceable bases diminishes. The soils represented by Points 7 and 8 produced high yields of vitamin C because the large amounts of bases which they contain caused high yields of dry matter.

The organic matter in the soil, as represented by the percentage loss on ignition, is shown (Fig. 11A) to bear little or no relationship to the concentration of vitamin C in the leaves of rye, although the amount of









organic matter was found to be directly related to the concentration of this vitamin in the leaves of oats. The favorable effect of the organic matter on the yield of vitamin C depends on its influence on the yield of dry matter. The higher amounts of organic matter are accompanied by greater amounts of replaceable bases, a fact which causes their effect on the yield of dry matter to be especially great.

The amount of nitrogen in the soil appears (Fig. 12A) to be inversely related to the concentration of vitamin C in the leaves. This relationship is directly opposite to that of the vitamin C content in oat leaves. The favorable effect of nitrogen on the yield of vitamin C (Fig. 12B) is due to the effect which the nitrogenous material, together with the replaceable bases which it maintains in the soil, exerts on the yield of dry matter.

The amount of weak-acid-soluble phosphorus in the soil is shown (Fig. 13A) to have a doubtful effect on the concentration of vitamin C. If Points 1 and 2 are ignored, the effect appears to be negative, but if Points 3 and 6 are ignored, the effect appears to be positive. The effect of phosphorus on the yield of vitamin C is a little more apparent. In the soils containing low amounts of replaceable bases, represented by Points 1 to 5, inclusive, it has a negative effect on the total yield. A similar effect is noted also in the group of soils containing the higher amounts of bases represented by Points 6 to 8, inclusive. The influence of phosphorus in the soil is particularly difficult to interpret. This problem will be discussed in detail in a future publication.

The effect of carbonates in the soil on the concentration of vitamin C is vague or lacking. On the other hand, the total yield of vitamin C from the calcareous soils represented by Points 1 to 6, inclusive, is shown (Fig. 14B) to be directly related to the carbonate content. This is due to the effect of the higher amounts of replaceable bases, which accompany the higher amounts of carbonate in these soils, on the yield of dry matter. Points 7 and 8 represent noncalcareous soils. They produce high yields of dry matter because of the large amount of replaceable bases which they contain, and consequently high yields of vitamin C.

A survey of all the data presented in the figures shows that, generally speaking, the magnitudes of the chemical properties of the soils which accompany the higher yields of dry matter lessen the concentration of vitamin C in the tissue of rye leaves. These effects are in direct opposition to those observed in oats grown during the previous autumn.

Previous reports by the authors have pointed out that the chemical properties of the soils which are related to the yield of dry matter, the concentration and yield of protein, and the concentration and yield of carotene exert the same general influence on both oats and rye even when these species are grown during different seasons of the year and on different groups of soils. The effects of soil properties on the formation and accumulation of vitamin C in cereal leaves show no such consistency. It does not follow, however, that definite relationships, yet unrecognized, do not exist.

SUMMARY

The chemical properties of eight soils in the vicinity of Midland, Douglas County, Kansas, were studied in relation to their effects on the accumulation of vitamin C (ascorbic acid) in the leaves of rye harvested at the jointing stage.

The relationships between the soil properties and the vitamin C content of the leaves are not always clearly evident. Generally, magnitudes of soil properties which favorably affect the yield of dry matter, lessened the concentration of vitamin C in the tissue.

Base-exchange capacity, total replaceable bases, replaceable calcium and magnesium, organic matter, and nitrogen were all negatively related to the concentration of vitamin C, whereas they were positively related in oats grown in the previous autumn.

The total yields of vitamin C in pounds per acre were governed by the yield of dry matter.

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NUTRITIVE VALUE OF FISH FROM MICHIGAN WATERS.

I. NICOTINIC ACID OF LAKE HERRING, CARP, COMMON SUCKERS, AND BURBOT[1]

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Although the retention of vitamins during the processing of meats and salt water fish has been reported [Schweigert, McIntire, and Elvehjem (1943); McIntire, Waisman, Henderson, and Elvehjem (1941); McIntire, Schweigert, Henderson, and Elvehjem (1943); Cheldelin and Williams (1943); Cheldelin, Woods, and Williams (1943); and Dann and Handler (1942)], investigation of the nutritive value of fresh water fish has not been included. This preliminary report is part of a study on the vitamin content of certain species of fish found abundantly in Michigan waters. Freezing, smoking, baking, and possible seasonal and environmental conditions have been studied as they affect the nicotinic acid content of lake herring *(Leucichthys artedi)*, common suckers *(Catostomus c. commersoni)*, carp *(Cyprinus carpio)*, and burbot *(Lota lota maculosa)*.

EXPERIMENTAL PROCEDURE

The lake herring used in the study were caught by commercial fishermen near Escanaba on Green Bay and near East Tawas or Bay Port on Saginaw Bay. The suckers were from near Cheboygan on Lake Huron, the carp from Monroe on Lake Erie and Saugatuck on Lake Michigan, and the burbot from Escanaba on Green Bay. The fish, packed in ice, were delivered within one or two days of the time of capture. After the fish were dressed, filleted, and smoked or frozen, nicotinic acid was determined by a modification of the Dann and Handler (1941) chemical method. Samples of nine to 27 fish of each species were analyzed.

To determine the loss of nicotinic acid during processing, one-half of the skinned fillet was analyzed raw; the other half was analyzed after baking or smoking. A fish for baking was laid on heavy waxed paper on a wire rack to prevent the loss of drippings and baked 15 minutes in a preheated gas oven at 176.7°C.(350°F.). One-half of the dressed herring or one-half fillet of sucker was prepared for smoking by immersion in 22½ to 25° salinometer brine[2] at 16°C.(60.8°F.) for 12 to 18 hours. The cured fish was laid on a wire rack in a smoke box and smoked for five to seven

[1] Approved by the director of the Michigan Agricultural Experiment Station for publication as Journal Article No. 749, n.s.

[2] Stock brine solution: 7½ lb. No. 1 fine salt, 2 lb. sugar, 1¼ lb. Prague powder (Griffeth's Laboratory, Chicago), 5 gallons water. This yields approximately an 80° salinometer brine which is diluted to 22½ to 25° as needed.

hours at 50 to 55°C.(122 to 131°F.) with smoke from mixed hardwoods. The entire fillet of herring or one-half fillet of sucker or carp or burbot was wrapped individually with parchment paper and heavy waxed paper and quick-frozen. Frozen fish were held at —17.8°C.(0°F.) in a commercial locker plant.

One-half of the herring fillet or cross sections of the carp, burbot, and sucker fillets were skinned and weighed for nicotinic acid analysis. A composite sample of roe from several fish was analyzed. The tissue was macerated with five to six times its weight in water for five to seven minutes in a Waring blendor. Three aliquots of the mixture weighing approximately 40 gm. each were introduced into 125-ml. Erlenmeyer flasks. To each was added 10 ml. of phosphate buffer, pH 7.0, containing 0.2 gm. of polidase-S.[3] The corked flasks were incubated for 16 to 18 hours at 45°C.(113°F.). The digested extracts were heated to boiling, cooled, and filtered through Whatman No. 4 filter paper.

Twenty milliliters of the digested filtrate were decolorized, using the procedure outlined by Dann and Handler (1941), with the following modifications. Concentrated hydrochloric acid was used to adjust the pH to 0.5 to 1.0, using methyl violet as an external indicator. Maximum decolorization was obtained by the addition of 0.9 to 1.1 gm. of powdered lead nitrate. The yellow color produced by the reaction of nicotinic acid, cyanogen bromide, and photol[4] was read in a Cenco photelometer with Corning filters No. 5113½ standard thickness, and 3060 giving maximum transmission at 400 millimicrons.

Two samples of the macerated tissue were dried overnight at 95 to 100°C.(203 to 212°F.), brought to room temperature in a desiccator, and weighed to determine the percentage of solids.

DISCUSSION OF RESULTS

Preliminary investigation in this laboratory showed that the extraction procedure influenced the nicotinic acid values obtained from fish. The results when paired samples of fish were used are given (Table 1). Incubation with papain and taka diastase at 45°C.(113°F.) or polidase at 37°C. (98.6°F.) for 16 to 18 hours at pH 4.5 gave values of 25 μg. per gm. of tissue. Duplicate samples digested with concentrated hydrochloric acid on a steam bath for one hour or incubated with polidase at pH 7 for 16 to 18 hours at a temperature of 45°C. or autoclaved 30 minutes with 15 pounds of pressure in mild or strong alkali showed mean values within the range of 40 to 50 μg. per gm. These results are in agreement with the statement made by Cheldelin and Williams (1942) that acid or alkali extraction was of no advantage in the analysis of milk or animal tissue.

The nicotinic acid content of lake herring, carp, common suckers, and burbot is presented (Table 2). The lake herring yield from 20 to 55 μg. of nicotinic acid per gram of tissue which is within the range of 32 to 40 μg. reported by Kringstad and Thoresen (1940) and Kodicek (1940) for

[3] Recommended by Clausen and Brown (1943) and obtained from Schwartz Laboratories, New York, New York.

[4] p-Methylamino phenol sulfate purchased from Merck and Company.

TABLE 1

Nicotinic Acid Values of Lake Herring Obtained by Various Extraction Procedures

Series 1		Series 2		Series 3			Series 4	
Enzyme digestion with polidase for 16-18 hr.		Enzyme polidase	Concentrated HCl on steam bath for 60 min.	Enzyme polidase pH 7.0, 45°C.	Autoclaved 30 min. with 15-lb. pressure		Enzyme digestion 16-18 hr. at 45°C.	
pH 4.5, 37°C.	pH 7.0, 45°C.	pH 7.0, 45°C.			Concentrated alkali	Mild alkali	Taka diastase and papain, pH 4.5	Polidase, pH 7.0
μg./gm.	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*
25.2	43.5	55.6	35.4	48.7	50.2	34.0	22.9	51.8
27.1	40.3	43.6	41.9	45.7	48.6	45.2	37.7	49.9
23.8	32.7	44.6	43.0	60.2	37.1	50.1	.....	.....
21.7	47.6	42.8	44.5	.....	.....	.....	.....	.....
.....	.....	50.6	32.9	.....	.....	.....	.....	.....
.....	.....	62.8	67.1	.....	.....	.....	.....	.....
Mean 24.4	41.0	50.0	44.2	51.5	45.3	43.2	25.3	50.9

TABLE 2

Nicotinic Acid Content of Fresh and Frozen Lake Herring, Carp, Burbot, and Common Sucker Fillets

Species and region of catch	Season caught	Period of frozen storage	Number of samples	Nicotinic acid		Solids	
				Mean	S.D.[1]	Mean	S.D.
Lake Herring		*mo.*		*μg./gm.*	*μg./gm.*	*pct.*	*pct.*
Saginaw Bay	Oct.-Nov. 1943	0	7	55.2	16.6	26.2	3.5
Saginaw Bay	Oct.-Nov. 1943	2½-3	20	45.8	8.5	25.5	3.0
Saginaw Bay	Oct.-Nov. 1943	9	20	37.7	7.5	22.7	1.6
Saginaw Bay	June 1944	0	18	31.5	5.6	20.6	1.5
Saginaw Bay	June 1944	2½-3	17	26.1	4.5	19.8	1.8
Saginaw Bay	Nov. 1944	0	20	23.2	5.5	18.7	1.9
Saginaw Bay (roe)	Nov. 1944	0	3	5.6	.....	37.4	.....
Green Bay	Feb. 1944	0	31	26.9	6.5	19.3	1.6
Green Bay	June 1944	0	18	22.7	8.4	21.7	2.1
Green Bay	June 1944	2½-3	15	21.4	6.0	22.1	1.7
Green Bay	Nov. 1944	0	18	22.6	4.5	19.2	1.7
Common Suckers							
Lake Huron	Mar.-Apr. 1944	0	27	12.7	2.4	21.2	0.9
Lake Huron	Mar.-Apr. 1944	3½	23	11.2	3.1	20.9	1.5
Lake Huron	Oct. 1944	0	9	11.4	1.8	21.4	0.7
Carp							
Lake Erie	May 1944	0	14	14.5	2.8	22.4	3.3
Lake Erie	May 1944	3½	13	15.5	4.3	21.9	1.8
Lake Michigan	Oct. 1944	0	20	14.7	4.2	20.2	1.3
Lake Michigan (roe)	Oct. 1944	0	3	13.3	.....	33.8	.....
Burbot							
Green Bay	Nov. 1944	0	5	16.4	.....	17.9	.....

[1] S.D. means standard deviation.

salt water herring. The nicotinic acid determined for common suckers was 13 μg. per gm. in March and 11 μg. in October, an insignificant difference.[5] Carp were found to have an average of 15 μg. per gm. in both May and October. Burbot averaged 16 μg. per gm. in November. Samples of lake herring and carp roe contained 6 and 13 μg., respectively. Thus the nicotinic acid content of lake herring was comparable to that of lean meat and chicken but that of suckers, burbot, carp, carp roe, and herring roe was low.

Saginaw Bay herring had a nicotinic acid value of 55 μg. per gm. in November, 1943; 32 μg. in June, 1944; and 23 μg. in November, 1944, a difference which was significant. Fish from Green Bay had mean values of 27 μg. per gm. in February, 1944, and 23 μg. per gm. in June and November, 1944, an insignificant variation. In comparing the fish from the two bays, a statistically significant difference ("t" = 5.0 with 34 D.F.) was noted in the nicotinic acid content of herring caught during June, 1944, but no difference was observed in those caught in November, 1944. The results indicate that season and environment may be influential factors in the nicotinic acid content of fish.

The weight-length ratio is used by the fisherman as an indication of the condition of the fish—the higher ratio indicating a fish with thick flesh. When the weight-length ratios were calculated on the drawn weight, the ratio for Saginaw Bay herring was 8.4 in October, 1943, for 52 fish; 7.1 for 34 fish in November, 1943; 4.5 for 63 fish in June, 1944; and 5.9 for 32 fish in November, 1944. Herring from Green Bay in June had a ratio of 4.6 for 31 fish and 5.9 for 21 fish in November, 1944. At the present time no explanation for this variation in weight-length ratio is known, but it is of interest that changes in nicotinic acid concentration paralleled changes in weight-length ratios. A variation similar to that found in the weight-length ratio was observed in the per cent solids (Table 2). Saginaw Bay herring had an average of 26 per cent solids in November, 1943, 21 per cent in June, and 19 per cent in November, 1944. Green Bay herring contained 19 per cent in February and November, 1944, and 21 per cent in June. Evidence of seasonal fluctuation has been observed by Raey, Cutting, and Shewan (1943) in the amount of glycogen and protein in fish. Raey and Cutting (1944) state that in salt water herring the seasonal variation in fat content may be from 0.4 to 22 per cent. Schweigert *et al.* (1943) report that feed and diet may be important factors in the thiamin content of pork muscle. All of the herring samples from Green Bay and some of those from Saginaw Bay in November, 1944, contained parasites tentatively identified as *Triaenophorus robustus*. Since numerous factors may have a relationship to the nicotinic acid content, analyses should be repeated over a period of years with fish from various locations and habitats before definite conclusions can be drawn.

Freezing losses of nicotinic acid as presented (Table 2) were insignificant when carp and two groups of lake herring were held in the frozen state for two and one-half to three months. The Saginaw Bay herring in June showed a slightly significant loss ("t"=2.98 with 33 D.F.). Herring

[5] In all cases "t" test was used to determine significance.

analyzed after nine months' storage retained an average of 67 per cent of the original nicotinic acid ("t"=3.63 with 25 D.F.). Paired data for suckers were not available in sufficient numbers to determine the loss during freezing. However, in the series of suckers analyzed fresh and frozen, the indications are that there is no significant loss of nicotinic acid during freezing.

Since nicotinic acid is heat stable and water soluble, it probably is not destroyed during cooking but is leached from the tissue. In fresh and frozen herring, fresh carp, and common suckers there was no significant loss of nicotinic acid during baking (Table 3) with the baking procedure

TABLE 3

Nicotinic Acid Concentration of Raw and Baked Lake Herring, Carp, and Common Sucker Fillets

Species and region of catch	Number of samples	Nicotinic acid				Solids			
		Raw		Baked [1]		Raw		Baked	
		Mean	S.D.[2]	Mean	S.D.	Mean	S.D.	Mean	S.D.
		μg./gm.	*μg./gm.*	*μg./gm.*	*μg./gm.*	*pct.*	*pct.*	*pct.*	*pct.*
Lake Herring									
Saginaw Bay (frozen)...	20	45.8	8.5	45.1	10.9	25.5	3.0	25.6	3.9
Green Bay (fresh).....	16	27.1	7.5	26.5	6.8	19.5	1.2	22.2	3.1
Common Suckers									
Lake Huron (fresh).....	14	12.7	2.4	13.0	3.2	21.2	0.9	21.4	1.4
Carp									
Lake Erie (fresh).....	14	14.5	2.8	15.6	4.5	22.4	3.3	25.8	3.2

[1] The sample weighed before baking and entire samples plus drippings were used for analysis.
[2] S.D. means standard deviation.

used in this laboratory where drippings were collected and analyzed with the flesh of the fish. If the nicotinic acid values calculated on the baked weight are compared with the corresponding raw values, there is an apparent increase in nicotinic acid since evaporation of moisture during baking caused an eight to 10 per cent loss of weight. Therefore, comparisons were calculated on the weight of the fish before baking. Cheldelin and Williams (1943) reported a two to seven per cent nicotinic acid loss in salmon and halibut during cooking. McIntire *et al.* (1943), who believe that the size of the cut and the method of cooking are influential factors, noted a 15-per cent loss of nicotinic acid in the cooking of meats.

Smoking, a common method of preserving fish, was used successfully in preserving lake herring and common suckers. During processing, the

loss of moisture caused an increase from 21 per cent solids in the raw fish to an average of 31 per cent in the smoked fish. The smoked herring had an average of 87 μg. of nicotinic acid per gm. of dried tissue and the corresponding raw fish had an average of 131 μg. per gm. or 66 per cent retention of the vitamin (Table 4). The smoked suckers in Series 1, which were cured 17 hours in 25° salinometer brine and averaged 33 μg. per gm. of (smoked) dry tissue, retained 55 per cent of the original nicotinic acid. Those in Series 2, which were cured 12 hours in 22½° brine and averaged 48 μg. per gm., had an average of 89 per cent retention. The longer brining period and the increased concentration of salt used in Series 1 probably caused more nicotinic acid to leach from the tissue, and therefore less of the original nicotinic acid was retained. Schweigert *et al.* (1943) found an average of 84 per cent retention during the curing of ham—a cut of meat which has less surface area but requires a longer curing and smoking time.

An average serving of lake fish as observed in this study would furnish approximately one-tenth of the daily nicotinic acid requirement of an average man.

SUMMARY AND CONCLUSIONS

The effect of environment, season of the year, and processing on the nicotinic acid content of lake herring, carp, burbot, and common sucker has been studied. Enzyme digestion and a modification of the Dann and Handler chemical method were used to determine nicotinic acid.

Lake herring from Saginaw Bay had an average of 55, 31, and 23 μg. of nicotinic acid per gm. of tissue in November, 1943, and June and November, 1944, respectively. Green Bay herring contained an average of 27 μg. per gm. in February and 23 μg. per gm. in June and November. No explanation other than season and environmental conditions was found to account for the highly significant differences in nicotinic acid content of herring studied.

Common suckers contained an average of 13 and 11 μg. of nicotinic acid per gm. of tissue in March and October, respectively. In May and October carp had 15 μg. per gm., burbot averaged 16 μg. per gm., carp roe had values of 13 μg. of nicotinic acid per gm., while herring roe contained 6 μg. per gm. In the species of fish studied there was wide individual variation.

Freezing and subsequent storage for two and one-half to three months caused insignificant loss of nicotinic acid in carp, common suckers, and two groups of lake herring.

When fresh or frozen fish were baked, there was no significant loss of nicotinic acid. Smoked herring and common suckers retained 66 and 72 per cent, respectively, of nicotinic acid in the processed fish.

The nicotinic acid content of fresh, frozen, and smoked lake herring is comparable to the nicotinic acid content of lean meat. The content of carp, burbot, and common suckers is slightly lower.

TABLE 4

Nicotinic Acid Content of Raw and Smoked Lake Herring and Common Suckers

Species and region of catch	Number of samples	Nicotinic acid								Solids			
		Raw (fresh)		Dry (fresh)		Smoked		Dry (smoked)		Fresh		Smoked	
		Mean	S.D.[1]	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
		μg./gm.	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*pct.*	*pct.*	*pct.*	*pct.*
Lake Herring[2]													
Green Bay	17	26.7	5.5	131	22	24.2	7.0	87	24	20.5	1.7	27.8	2.1
Common Suckers[3]													
Lake Huron	8[4]	12.9	1.7	60	10	10.1	3.5	33	12	21.7	0.8	30.4	3.8
Lake Huron	8[5]	11.3	1.7	54	8	16.8	2.4	48	7	20.6	0.8	34.9	1.9

[1] S.D. means standard deviation. [2] Filleted after smoking. [3] Filleted before smoking. [4] Cured17 hours in 25° salinometer brine. [5] Cured 12 hours in 22½° brine.

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DETERMINATION OF SURFACE AREA OF DEHYDRATED EGG POWDER

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Reactions involving dehydrated egg powders and various gases, especially oxygen and carbon dioxide, are being studied in this laboratory. This work necessitated determining the surface areas of the egg powders, which was done by two methods. The first method, called the low temperature-gas adsorption method, was chosen because it is probably the most reliable for this purpose. It is not, however, well adapted to rapid measurements such as might be required in routine determinations. For this reason, the permeability method of determining surface areas was also investigated.

The latter method, in contrast to the adsorption method, requires only simple equipment and is well adapted to measurements where large numbers of samples are involved. It is recognized, of course, that the low temperature-adsorption method and the permeability method do not measure the same surface areas except for smooth particles. Nevertheless it was considered of value to investigate the applicability of the permeability method to egg powders. Advantages would be gained even though the method might be found valid only for the determination of relative areas rather than absolute areas.

LOW TEMPERATURE-ADSORPTION ISOTHERM METHOD

The low temperature-adsorption isotherm method—Brunauer, Emmett, and Teller (1938)—is probably the most reliable method known for determining the surface area of fine powders. Since the theoretical basis and experimental details have been described in numerous places they will not be repeated here. The reader is referred particularly to recent discussions by Emmett (1942) and Brunauer (1943).

The gases adsorbed in the present work were argon and nitrogen. The argon was 99.6 per cent pure and was used as received. The nitrogen was a commercial oil-pumped product and was subjected to a final purification by passage over copper powder [at 450°C.(842°F.)], Ascarite, and P_2O_5. Adsorption measurements were made with the sample vessel, containing from 15 to 60 grams of egg powder, immersed in a bath of liquid nitrogen (77°K.).

The dehydrated eggs studied included six commercial spray-dried[2] samples and three lyophilized samples prepared in this laboratory. Before being used for surface determinations they were evacuated for at least three of four days at room temperature [25°C.(77°F.)]. At the end of the

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evacuation period a McLeod gauge, connected to the sample vessel (with the pumping system cut off), read about 10^{-4} mm. Hg. This evacuation procedure is considered sufficient to remove adsorbed gases and vapors likely to interfere with surface measurements because no change in surface area could be detected on samples for which the evacuation at room temperature was continued for periods up to 10 days. A further evacuation of Sample C81R for 16 hours at 40°C.(104°F.), followed by six hours at 100°C.(212°F.), resulted in a decrease in surface area of the same order as the experimental error (five per cent).

Typical isotherms for the adsorption of nitrogen and argon by dehydrated eggs (Fig. 1) are of the standard S-shaped variety typical of

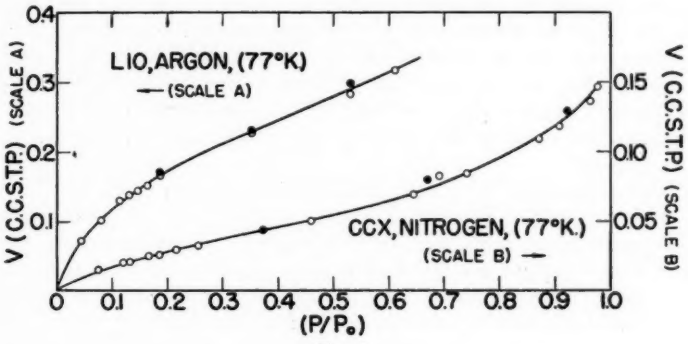


FIG. 1.

multimolecular adsorption. The theory of Brunauer, Emmett, and Teller (1938), hereafter referred to as the BET theory, predicts that for low relative pressures (P/P_o), the quantity $P/[V(P_o-P)]$ is a linear function of P/P_o. Here P and P_o are, respectively, the absolute and condensation pressures of the gas and V is the volume (at standard conditions) of gas adsorbed. The data (Fig. 1) at relative pressures less than 0.3 are seen (Fig. 2) to exhibit such a linear relationship. The volume of gas required to form a unimolecular layer on the surface of the adsorbent can be calculated from the slope and zero pressure intercept of the straight-line plot of Fig. 2 by the BET theory. From the volumes thus obtained and the average cross-sectional area of the adsorbed molecules the surface area of the adsorbent can be calculated.

Values for the surface area of dehydrated eggs obtained in the manner outlined above (Table 1) are based on the assumption that the average cross-sectional area occupied by an adsorbed molecule is 15.4×10^{-16} sq. cm. for nitrogen and 13.6×10^{-16} sq. cm. for argon, Livingston (1944). Also shown in the table are the porosities to which the samples were packed

[2] One of the spray-dried samples was prepared by a special process in which the liquid egg was concentrated until the solid content was 35.7 per cent before being introduced into the spray drier. A sample of this egg powder was furnished the authors through the kindness of Capt. George Gelman, U. S. Army, Q.M.C., Subsistence Laboratory, Chicago, Illinois. Data for these eggs are given under laboratory sample "H" (Table 1).

during the adsorption measurements. The porosity, ϵ, is defined as the fraction of the total volume of a packing which is void.

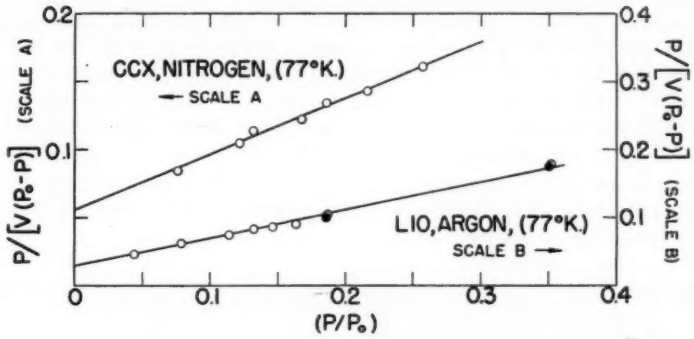


FIG. 2.

The specific surface areas of dehydrated eggs vary over a range of about sevenfold, the spray-dried samples having, in general, smaller surface areas (Table 1). The nitrogen and argon values for the surface area of the same sample differ by less than 20 per cent. This difference is of the same order of magnitude as the uncertainty in the cross-sectional area of the adsorbed molecules. Repeat measurements of the area of the egg powders agreed within five per cent.

TABLE 1

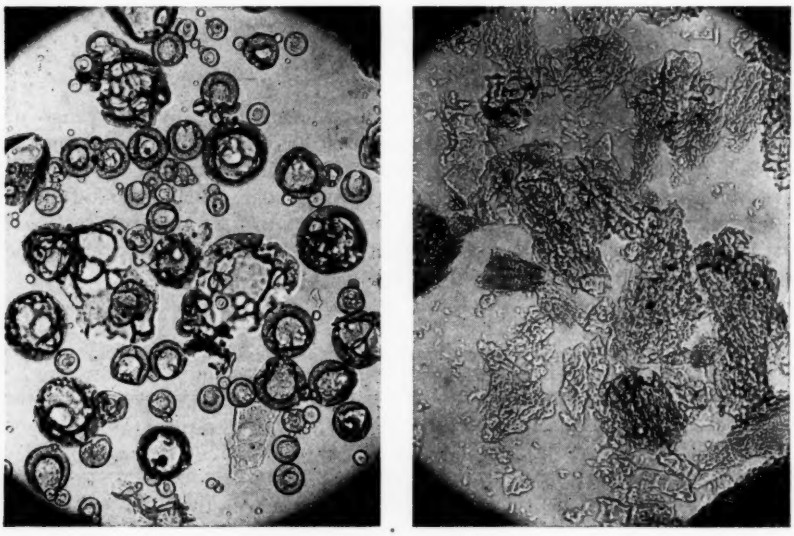
Summary of Specific Surface Areas of Various Samples of Spray-Dried and Lyophilized Egg Powders[1]

Description of sample		Porosity	Adsorbed gas	Specific surface (square meters/gram)
Laboratory No.	Method of drying			
CCX	Spray dried	.445	N_2	.14
CCX	Spray dried	.445	A	.13
C48R	Spray dried	.400	N_2	.10
C48R	Spray dried	.400	A	.12
C77R	Spray dried	.46	N_2	.43
C77R	Spray dried	.46	A	.41
C81R	Spray dried	.505	A	.12
C86R	Spray dried	.559	A	.41
H	Spray dried	.425	N_2	.21
H	Spray dried	.425	A	.21
L10	Lyophilized	.699	N_2	.75
L10	Lyophilized	.699	A	.68
LN11	Lyophilized	.629	N_2	.51
LN11	Lyophilized	.629	A	.45
LO	Lyophilized	.580	N_2	.83

[1]Areas calculated from the low temperature-adsorption isotherms for nitrogen and argon.

The areas shown (Table 1) do not represent an intrinsic property of dehydrated eggs but rather reflect the conditions under which the eggs were dried. Particles of spray-dried eggs are more or less spherical in shape. Some of the particles are solid and others contain voids. On the other hand, lyophilized eggs when removed from the drier are in the form of a sponge-like mass. The particles obtained when the mass is broken apart are plate-like and of irregular outline. Presumably, dehydrated eggs with surfaces greater or less than those shown (Table 1) can be obtained by a proper choice of drying conditions.

The nature of the particles of dehydrated eggs is evident in the photomicrographs (Fig. 3), which show very clearly the wide variations in size and shape of the particles.



Sample C86R, spray-dried, 150× Sample LO, lyophilized, 150×

FIG. 3. Photomicrographs of particles of dehydrated eggs.

PERMEABILITY METHOD

The permeability method was used essentially as described by Lea and Nurse (1939) with the exception that, instead of air, commercial nitrogen was used after it was passed through a P_2O_5 tube to remove residual water. The permeability cell was made of one-inch inside diameter brass tubing. A brass disc perforated with about 150 holes, each $\frac{1}{32}$ inch in diameter, was soldered into the tube to support the sample. A piece of filter paper of negligible specific surface area was placed on the disc before the sample was placed in the tube. The sample was compacted with an aluminum plunger. The plunger was graduated in millimeters in order to measure the thickness of the sample. Permeability measurements were made for each sample of dehydrated egg powder at several different degrees of

packing or porosities. All experiments were performed at room temperature (25 ± 3°C.).

The specific surface area per gram was calculated from the following equation, Carman (1938):

$$S_g = 14\sqrt{(1/K\nu)\epsilon^3}/[d(1-\epsilon)] \qquad \text{(c. g. s. units)}.$$

In this equation, K = permeability, representing rate of linear flow for unit hydraulic gradient, ν = kinematic viscosity, ϵ = porosity, and as above, d is the density of the dehydrated eggs equal to 1.14 grams per cubic centimeter.

TABLE 2

Variation of Specific Surface With Porosity[1]

Laboratory No.	Porosity	Specific surface (square meters/gram)
CCX	.552	.168
	.543	.179
	.517	.204
	.467	.226
C48R	.585	.074
	.569	.093
	.553	.108
	.534	.124
	.510	.149
	.493	.168
	.469	.197
C77R	.661	.284
	.649	.332
	.636	.388
	.622	.505
	.606	.578
C81R	.587	.159
	.572	.175
	.555	.192
	.538	.208
	.498	.252
L10	.781	.344
	.762	.386
	.745	.450
	.721	.560
	.698	.674
LN11	.711	.403
	.692	.515
	.666	.562
	.628	.825

[1]Areas calculated from permeability measurements.

Typical results for several samples of dehydrated egg powder (Table 2) reveal that the important fact is the strong dependence of surface area per gram on the porosity of the sample in the permeability tube. If it is assumed that S_g is a constant characteristic of the particular sample of dehydrated egg powder, then S_g should be independent of the porosity at which the permeability measurements were made, since the equation given

above contains a term designed to account for porosity variations. The work of Carman (1938) has shown this term is adequate to account for porosity variations for the materials studied by him.

A behavior similar to that found for dehydrated eggs has been reported for Portland cement powder, American Society for Testing Materials (1942). It has been suggested, in this instance, by Rigden and Lea (1943) that the anomalous behavior arises from nonuniform packing of the powder. Rigden (1943) has reported data for mineral powders and cement which are apparently free from porosity effects. In this work the powders were packed in the permeability tube in a series of "steps" about two millimeters deep until the desired thickness of powder was obtained.

The use of layers of egg powder of various thicknesses in the range 0.75 to 4.78 cm. was investigated for dehydrated eggs. In some experiments sufficient egg powder was placed in the permeability tube to obtain the desired thickness in one compaction. In other trials the powder was compacted in layers a few millimeters thick. No difference in the dependence of the surface area on the porosity was found.

To test the apparatus and technique used in the present work for other possible sources of the anomalous behavior found for dehydrated eggs, measurements were made for diatomaceous earth (Celite) and powdered pumice. For these materials S_g was found to be constant independent of the porosity. It is concluded therefore that the anomalous dependence of S_g on the porosity is related to the nature of the egg particles.

DISCUSSION

It is well recognized, of course, that in general the surface areas determined by the adsorption method and by the permeability method may or may not be the same, the surface area available to the gas in a permeability measurement being less than, or equal to, the area available determined by low-temperature adsorption.

It is of interest therefore to compare the surface areas of egg powders as determined by these two methods. Since the permeability data given (Table 2) cover a range of porosities, it is possible, for most samples, to make the comparison at the porosity employed in the adsorption measurements. With one exception the adsorption measurements were made at a single porosity only. In that case, for Sample C77R, identical surface areas were obtained by the adsorption measurement for porosities of 0.461 and 0.508.

When the surface areas of the egg powders shown (Tables 1 and 2) are compared, it is found that in general the areas obtained by the permeability method exceed those obtained by the adsorption method. In only one instance, for Sample L10, are the areas about the same.

Rigden (1943) has stated that with fine powders more accurate results should be obtained at low porosities because channeling and bridging in the packed mass of particles is less likely. For the egg powders, however, the surface areas for the low porosities are even less compatible with the adsorption data.

In view of the anomalous dependence of the permeability measurements on porosity and the lack of consistency between the adsorption and perme-

ability measurements, it appears that permeability measurements are not suitable for the determination of the surface areas of egg powders to better than order-of-magnitude accuracy.

In seeking an explanation of the failure of the permeability method for use with dehydrated egg powders several possibilities have been considered. For the most part they have been concerned with possible alterations in the path of flow of the gas in the egg powder as a function of the porosity. While it seems reasonable to account for an increase in the apparent surface area of the powder with decreasing porosity on this basis, it seems impossible to reconcile the fact that the permeability measurements at low porosity give a surface area greater than that obtained by low temperature-adsorption measurements. On the other hand, the source of the difficulty may be the equation used to calculate the surface area of the particles from the permeability measurements. Despite the fact that Carman (1938) showed by experiment that the equation used in the present work was applicable to particles of intricate geometry, it should not be overlooked that the particles of known area tested by Carman are much larger than the particles of egg powder and other materials of unknown area to which the equation has been applied.

SUMMARY

The low temperature-gas adsorption method and the permeability method have been used to determine the surface areas of representative spray-dried and lyophilized egg powders. The surface areas found by the low temperature-gas adsorption method range from about 0.1 to 0.8 square meter per gram. Lyophilized powders have larger areas than spray-dried powders.

The permeability method is shown to be unsatisfactory for the accurate determination of the surface areas of dehydrated egg powders because of an anomalous dependence of surface area on the porosity to which the eggs are packed in the permeability cell.

ACKNOWLEDGMENT

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RELATIONSHIPS BETWEEN DRY WEIGHT, PROTEIN, AND VITAMINS IN LEAVES OF CEREALS[1]

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Cereal grasses have achieved a new prominence during the past few years as a source of protein and vitamins for human consumption. When cereals are grown for these purposes, they are harvested at, or just before, the jointing stage since at this stage of physiological development their leaves contain unusually high concentrations of digestible protein and vitamins. The concentrations of these substances change rapidly during the growth of the plants, and it is not unusual for the food value of the tissue to diminish during a period as short as two or three days to such a low point that the crop cannot be used. It is, therefore, of great importance that rapid tests be carried out to determine the suitability of the tissue as a source of protein and vitamins before harvests are made.

The purpose of the present paper is to describe certain relationships between the yield of dry matter, the yield and concentration of protein, of carotene, and of vitamin C (ascorbic acid), in the hope that the results will be of value in determining rapidly the food values of oats and rye when grown as sources of these substances.

MATERIALS AND METHODS

Samples of oats and rye at the jointing stage were obtained from the harvests of a group of 14 fields in the vicinity of Midland, Douglas County, Kansas. The material was quickly dried in an Arnold hot-air dehydrator and the chemical determinations were made immediately. The fields were from 20 to 40 acres in extent, and the entire harvest from each field was used in calculating the yield of dry matter in pounds per acre. The nitrogen content of the tissue was determined by the Kjeldahl procedure modified to include the nitrogen of nitrates as recommended by the Association of Official Agricultural Chemists (1935). The carotene was determined by the method described by Buxton and Dombrow (1938), except that methyl alcohol was substituted for ethyl alcohol and the heptane portion was concentrated on a hot plate in a flask with a vertical condenser instead of by vacuum distillation under nitrogen. The ascorbic acid content was determined by triturating one gram of dry tissue with 25 milliliters of an acid mixture containing two per cent of metaphosphoric acid and three per cent of trichloroacetic acid. One milli-

[1] The expenses incurred during this study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

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TABLE 1

Yield and Composition of Leaves of Oats

File No.	Field No.	Date	Point No.	Dry matter per acre	Crude protein (N x 6.25)		Carotene		Ascorbic acid	
				lb.	*pct.*	*lb./acre*	*mg. pct.*	*lb./acre*	*mg. pct.*	*lb./acre*
315	2A	Oct. 8, 1940	1	221	25.81	57.04	41.8	.092	425	0.939
317	2B	Oct. 11, 1940	2	304	28.88	87.80	49.8	.151	486	1.477
319	19	Oct. 17, 1940	3	200	18.80	37.60	33.3	.067	389	0.778
320	11E	Oct. 18, 1940	4	200	22.00	44.00	.....	.....	.....	.....
321	12	Oct. 21, 1940	5	389	24.06	93.59	45.8	.178	472	1.836
318	14B	Oct. 23, 1940	6	253	23.38	59.15	37.0	.094	361	0.913

TABLE 2

Yield and Composition of Leaves of Rye

File No.	Field No.	Date	Point No.	Dry matter per acre	Crude protein (N x 6.25)		Carotene		Ascorbic acid	
				lb.	*pct.*	*lb./acre*	*mg. pct.*	*lb./acre*	*mg. pct.*	*lb./acre*
332	9	Apr. 10, 1941	1	144	18.00	25.92	32.0	.046	372	0.54
334	10	Apr. 11, 1941	2	221	16.75	37.02	29.0	.064	338	0.75
335	20	Apr. 12, 1941	3	379	21.88	82.93	33.0	.125	387	1.47
336	19B	Apr. 13, 1941	4	500	16.94	84.70	28.3	.142	291	1.46
337	22	Apr. 14, 1941	5	298	16.94	48.81	25.8	.077	291	0.87
338	11	Apr. 17, 1941	6	947	21.94	207.77	32.0	.303	226	2.14
339	15A	Apr. 20, 1941	7	1430	23.56	336.91	37.8	.541	274	3.92
340	15B	Apr. 20, 1941	8	1950	20.50	403.85	32.3	.636	274	5.40

liter of the filtrate was titrated with a standard solution of the sodium salt of 2,3-dichlorophenolindophenol.

The data for six samples of oat leaves are presented (Table 1), and for eight samples of rye leaves (Table 2). The file numbers refer to the original samples permanently preserved in the Department of Botany and Plant Pathology at Michigan State College. The point numbers permit the data represented in the figures to be identified in the tables.

The data are plotted in the figures numbered 1 to 22; the curves in these figures were determined by the method of least squares. Three different regression curves may be drawn depending on which variable is considered as the known value and on which variable is to be minimized. In the present study the values represented on the ordinate are considered to be the known values, from which the values on the abscissa are predicted. The procedure adopted, therefore, was the one which minimized the spread of the points with respect to the abscissa. It is not intended that the regression curves in the figures represent exact, quantitative relationships. They are intended to represent trends only. It would be difficult, and perhaps impossible, to determine exact, quantitative relationships in material grown under field conditions. Although these general trends could be determined by inspection, the regression curves were calculated to lessen the personal errors which the drawing of freehand curves would entail.

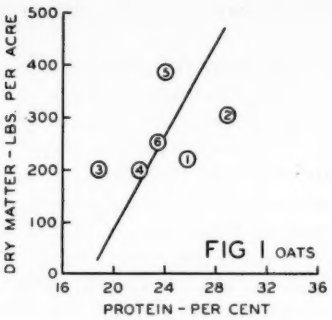
DISCUSSION

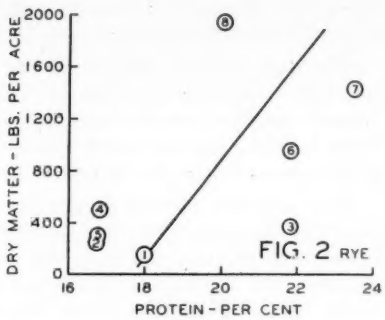
Yield of Dry Matter: The yield of dry matter is necessarily small when cereals are harvested at the jointing stage, a fact which makes it necessary to consider the total yield of protein and vitamins as well as their concentration in the tissue.

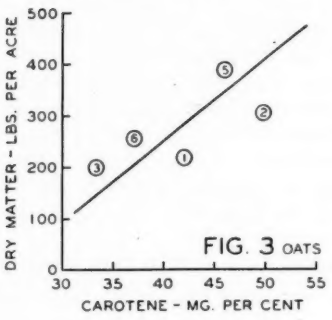
Figures 1 and 2 show that the higher yields of dry matter are associated with the higher concentrations of protein in the leaves. The data presented in Figs. 3 and 4 indicate that the higher concentrations of carotene also are associated with the higher yields of dry matter. Figure 5 indicates that the higher yields of dry matter of oats, grown during the autumn of 1940, contained the higher concentration of ascorbic acid, while Fig. 6 shows that the opposite was true for rye grown during the spring of 1941. The conditions which affect the concentration of ascorbic acid in plant tissue are especially complex; it is apparent, however, that it is not intrinsically related to the yield of dry matter.

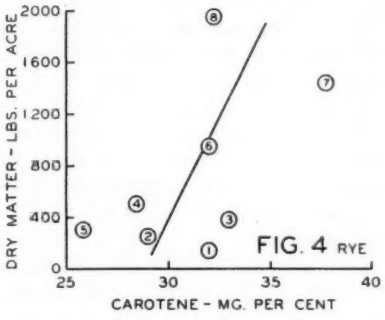
The yield of dry matter is very important in governing the total yield of protein, carotene, and ascorbic acid in pounds per acre. Figures 6 to 12, inclusive, show the very close relationship that exists between the yield of dry matter of both oats and rye and the yields of each of these substances.

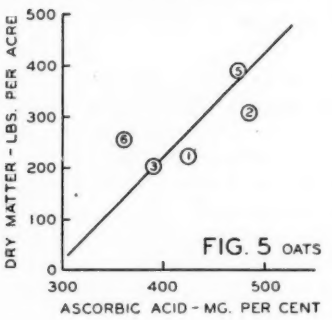
Percentage of Crude Protein: The necessity for quickly determining the quality of the cereal grass before it is harvested precludes the possibility of determining each of the vitamins. It is of considerable interest to discover how accurately the protein content alone indicates the general quality of the leaves. Figures 13 and 14 indicate that the percentage of protein varies directly as the concentration of carotene. The data in Fig.

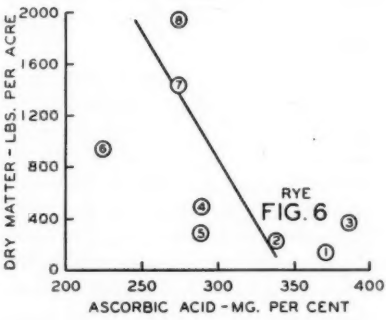

DRY MATTER - LBS. PER ACRE
500
400
300
200
100
0
16
20
24
28
32
36
PROTEIN - PER CENT
FIG 1 OATS

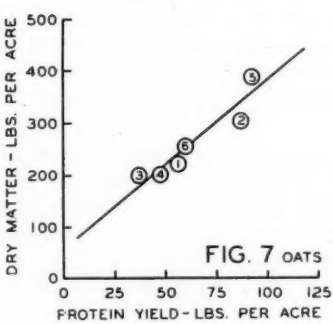

DRY MATTER - LBS. PER ACRE
2000
1600
1200
800
400
0
16
18
20
22
24
PROTEIN - PER CENT
FIG. 2 RYE

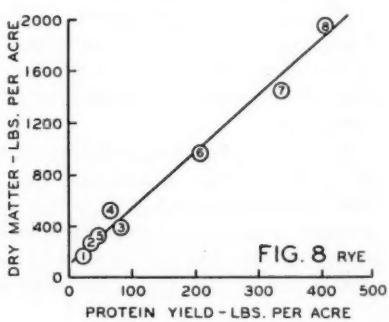

DRY MATTER - LBS. PER ACRE
500
400
300
200
100
0
30
35
40
45
50
55
CAROTENE - MG. PER CENT
FIG. 3 OATS


DRY MATTER - LBS. PER ACRE
2000
1600
1200
800
400
0
25
30
35
40
CAROTENE - MG. PER CENT
FIG. 4 RYE


DRY MATTER - LBS. PER ACRE
500
400
300
200
100
0
300
400
500
ASCORBIC ACID - MG. PER CENT
FIG. 5 OATS


DRY MATTER - LBS. PER ACRE
2000
1600
1200
800
400
0
200
250
300
350
400
ASCORBIC ACID - MG. PER CENT
RYE
FIG. 6


DRY MATTER - LBS. PER ACRE
500
400
300
200
100
0
0
25
50
75
100
125
PROTEIN YIELD - LBS. PER ACRE
FIG. 7 OATS


DRY MATTER - LBS. PER ACRE
2000
1600
1200
800
400
0
0
100
200
300
400
500
PROTEIN YIELD - LBS. PER ACRE
FIG. 8 RYE

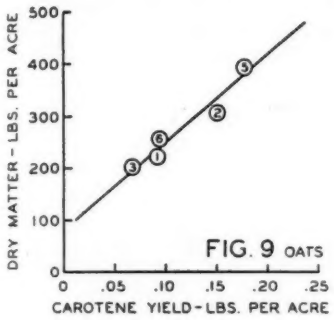


FIG. 9 OATS

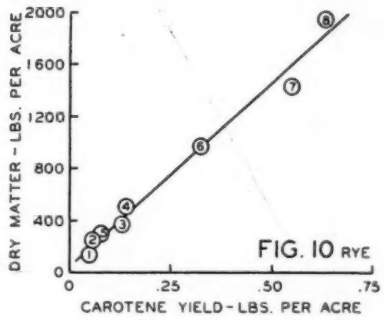


FIG. 10 RYE

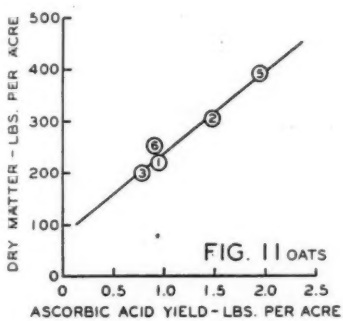


FIG. 11 OATS

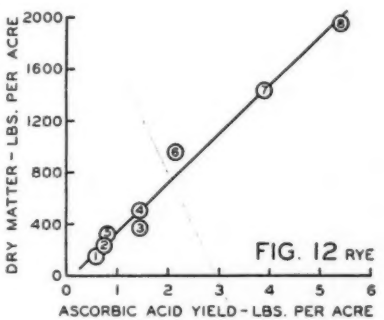


FIG. 12 RYE

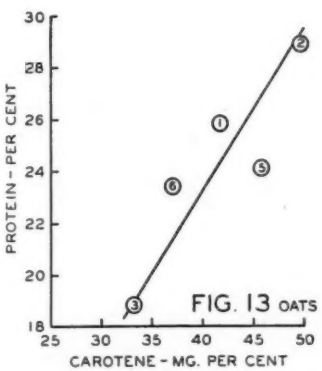


FIG. 13 OATS

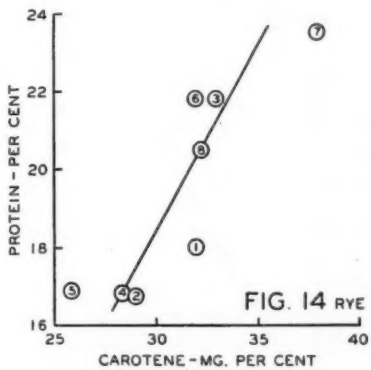
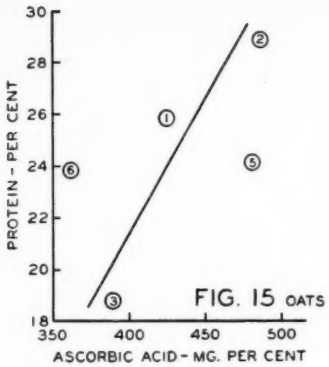
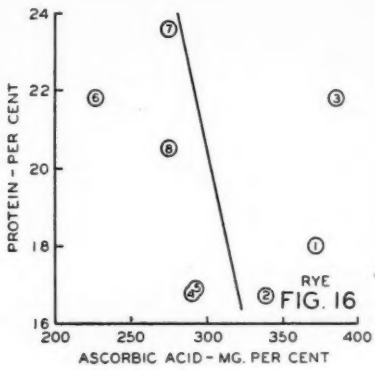
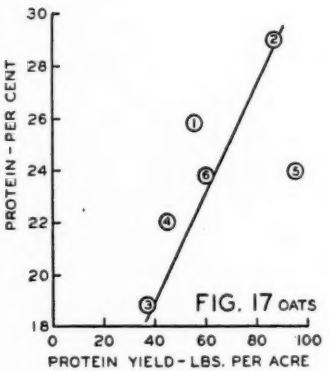
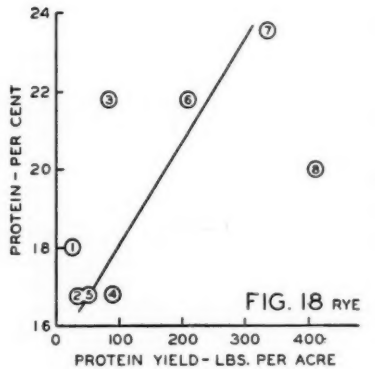


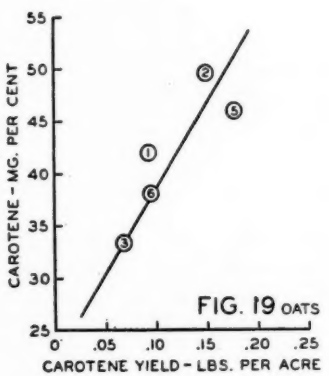
FIG. 14 RYE

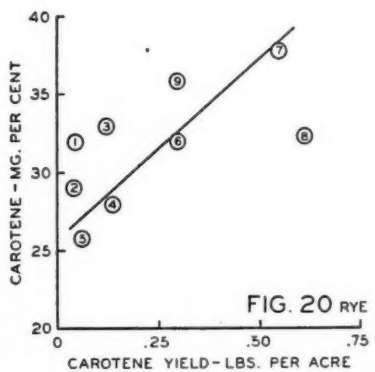

PROTEIN - PER CENT
30
28
26
24
22
20
18
350
400
450
500
ASCORBIC ACID - MG. PER CENT
FIG. 15 OATS


PROTEIN - PER CENT
24
22
20
18
16
200
250
300
350
400
ASCORBIC ACID - MG. PER CENT
RYE
FIG. 16

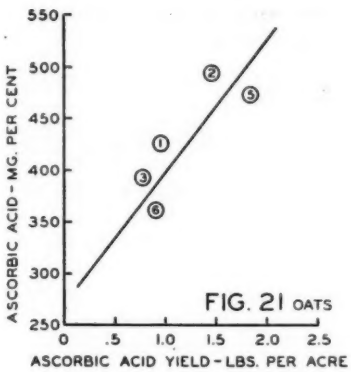

PROTEIN - PER CENT
30
28
26
24
22
20
18
0
20
40
60
80
100
PROTEIN YIELD - LBS. PER ACRE
FIG. 17 OATS

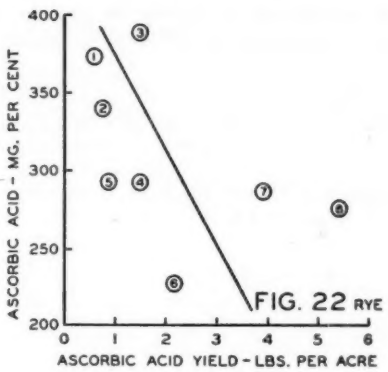

PROTEIN - PER CENT
24
22
20
18
16
0
100
200
300
400
PROTEIN YIELD - LBS. PER ACRE
FIG. 18 RYE


CAROTENE - MG. PER CENT
55
50
45
40
35
30
25
0
.05
.10
.15
.20
.25
CAROTENE YIELD - LBS. PER ACRE
FIG. 19 OATS


CAROTENE - MG. PER CENT
40
35
30
25
20
0
.25
.50
.75
CAROTENE YIELD - LBS. PER ACRE
FIG. 20 RYE

15 show that the percentage of protein varies directly with the concentration of ascorbic acid in the leaves of oats grown during the autumn, but Fig. 16 shows that this relationship is negative, or possibly absent, in the leaves of rye grown in the spring. This is an example of the sensitivity of the processes affecting the formation of ascorbic acid by plant cells to factors, both known and unknown, in the environment.





Percentages and Total Yields of Components: It has been stated above that the yields of dry matter are important in determining the yields of protein, carotene, and ascorbic acid. Since the total yields of these substances are affected also by their concentrations in the tissue, it is necessary to know which of these factors is the most important in controlling their total yields. Figures 17 and 18 show that the percentage of protein in the tissue varies directly with the total yield of protein, although it is apparent from a comparison of the distribution of the points in these figures with their distribution in Figs. 7 and 8 that the percentage of protein is a less reliable indication of the total yield of protein than is the yield of dry matter. Figures 20 and 21 show that the concentration of carotene also varies directly with the yield of carotene, but, as in the instance of protein, its concentration is not as reliable as the yield of dry matter for predicting the total yield of carotene. The concentration of ascorbic acid in the leaves of oats is shown in Fig. 21 to vary directly as its yield, while the opposite is true for rye. In both instances, however, the yield of dry matter, as shown by Figs. 11 and 12, is directly related to the yield of vitamin C.

SUMMARY

The quantitative relationships between the dry matter, protein, carotene, and vitamin C, in the leaves of oats and rye harvested at the jointing stage, are discussed and represented graphically by regression curves.

Higher yields of dry matter are associated with the higher concentrations of protein and carotene, but the yields of dry matter are not intrinsically related to the concentration of ascorbic acid.

The yields of dry matter determine the total yields of protein, carotene, and ascorbic acid.

The percentage of protein in the leaves of both oats and rye varies directly with the concentration of carotene but sometimes directly, and sometimes indirectly, with the concentration of ascorbic acid.

The concentrations of protein and of carotene vary directly as their total yields, while the concentration of ascorbic acid varies sometimes directly, and sometimes indirectly, with its total yield.

The yields of dry matter, of both oats and rye, are more important in governing the yields of protein, carotene, and ascorbic acid than are the concentrations of these substances in the tissue.

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PREVALENCE OF *CLOSTRIDIUM BOTULINUM* IN SOILS OF CENTRAL NEW YORK STATE[1]

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Recent occurrence of botulism in New York state justifies renewal of interest in the presence of *Clostridium botulinum* in soil of this district, especially since there appears to be little apprehension of the disease in this part of the country as compared with the west coast. In addition, little attention has been given to soils of this state apart from the work of Meyer and Dubovsky (1922a) which involved the collection of but few samples from a very restricted area of the state.

Although van Ermengem (1897) discovered *Clostridium botulinum* to be the causative organism of botulism as early as 1896, he failed to isolate it from a natural source. The isolation of a strain of the organism from animal excreta shortly after directed attention to the bacteriological analysis of feces and autopsy material in attempts to establish the enteric tract as being the natural habitat of the organism. It was not until it became evident that substantial proof of this was lacking that attention was diverted to the soil as being a possible source of the organism. Little was done, however, in regard to determining the presence of *Clostridium botulinum* in the soil until 1918 when Burke (1919) made a brief survey of four areas of California and was able to present proof of the natural occurrence of the organism. Since then numerous investigators have supplied abundant evidence that *Clostridium botulinum* has its natural habitat in the soil of the United States of America—Tanner and Dack (1922), Coleman (1922), Meyer and Dubovsky (1922b), Hall and Peterson (1924), Damon and Payabal (1926); of Alaska and Canada—Dubovsky and Meyer (1922b); of Great Britain—Leighton and Buxton (1928-29), Meyer and Dubovsky (1922c); of several European countries—Meyer and Dubovsky (1922c); and of the Hawaiian Islands and China—Schoenholz and Meyer (1922).

METHOD OF STUDY

In this study, samples of cultivated and virgin soils[2] and dusts were collected in the five central counties of New York state. At the time of collection, records were made as to the type and series of soil, crop, or type of woodland. Hydrogen-ion concentrations were determined and analyses for *Clostridium botulinum* were made on the basis of the formation of toxin by this organism in a favorable medium. The type of the organism was determined by toxin-antitoxin tests.

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Home Economics in the Graduate School of Syracuse University, December 1944.

[2] The term, virgin soil, is defined as forest areas believed to have been uncultivated in the past.

Soil specimens of 250 to 300 grams were collected between May and September, 1944, when long periods of dry weather ensured the comparative dryness of the soil. Because the presence of spores may be related to the water content of the soil, a record of the rainfall in the region of investigation was obtained from the weather bureau. With the exception of May and June, the precipitation was less during this investigation than normal and the total rainfall for the whole period was slightly less than normal. No soil collection was made until at least three or four days following a rainfall.

Rainfall (inches)	May	June	July	Aug.	Sept.	Total
Normal	3.00	3.87	3.68	2.86	2.72	16.13
1944	3.19	5.89	1.43	2.65	2.18	15.34

Specimens collected included muck, clay, sandy loam, silt loams, the latter predominating, and soil from woodland bearing coniferous, deciduous, and mixed growth. Samples were obtained from the surface layers of the soil to a depth of not more than six inches. Dust was collected from the floors and ledges of barns and other farm buildings. The samples were obtained with sterile spoons and placed in airtight, metal containers previously sterilized at 180°C.(356°F.) for three hours. All specimens were used within 24 hours of collection.

Thirty-gram portions weighed under aseptic conditions, were placed in 250-ml. Erlenmeyer flasks containing 30 ml. of sterile salt solution, pH 7. The flasks were then heated in a water bath for one hour at 80°C.(176°F.). Ninety ml. of a sterile, beef-heart, peptic-digest broth, Dubovsky and Meyer (1922a), was added to the soil-salt solution mixture and the two liquids mixed by careful rotation to avoid aeration. This medium was selected as the most favorable for toxin formation after comparison with three commercial media. Before adding the broth, precaution was taken to eliminate dissolved oxygen by boiling. The surface of the fluid was flooded with sterile mineral oil to a depth of five centimeters and the flasks were then replugged and incubated at 37°C.(98.6°F.) for 10 days. Ten ml. of the supernatant fluid was removed by pipette and placed in a sterile test tube which was centrifuged at 4,000 revolutions per minute for 30 minutes. Two ml. of this supernatant fluid was fed to a standard guinea pig (250 to 300 gm.) while in the post-absorptive state, preliminary experiments having indicated that this condition was the best time for feeding. No signs of regurgitation were noted at any time during the experiments. The death of the animal within four days suggested the presence of *Clostridium botulinum* in the soil. The strain was then typed by toxin-antitoxin tests. The antitoxin received by the surviving animals indicated the type of *Clostridium botulinum* present in the soil sample under investigation.

EXPERIMENTAL RESULTS

Of the 283 samples collected, 238 were soil and 45 were dust specimens. Of the soils collected 178 were obtained from cultivated fields and 60 were from virgin-forest areas; a summary of the source of the specimens collected is presented (Table 1).

Of the total 283 samples tested 33, or 11.7 per cent, were found to be toxic; 26, or 78.7 per cent, of these yielded type A toxin; five, or 15.1 per

TABLE 1

Source of Material Used in Determination of Clostridium botulinum in Soils of Central New York State

County	Cultivated soils	Virgin soils	Barn dust	Total
Onondaga	93	10	16	119
Oswego	12	18	8	38
Madison	31	21	4	56
Cortland	17	5	5	27
Cayuga	25	6	12	43
Total	178	60	45	283

TABLE 2

Relative Toxicity of Different Types of Collected Material

Soil	Number of samples	Toxic samples	Toxicity	Type of toxin		
				A	B	A & B
			pct.			
Cultivated	178	26	14.6	21	4	1
Virgin	60	3	5.0	3	....	....
Barn dust	45	4	8.8	2	1	1
Total	283	33	11.7	26	5	2

TABLE 3

Type of Cultivated Soil in Relation to Toxicity

Type of soil	Number of samples	Toxic samples	Toxicity	Type of toxin		
				A	B	A & B
			pct.			
Muck	2	....	.....	....	....	....
Clay	3	....	.....	....	....	....
Sandy loam	12	....	.....	....	....	....
Silt loam	161	26	16.4	21	4	1
Total	178	26	16.4	21	4	1

TABLE 4

Toxicity in Relation to pH Values of Soils and Dusts

Source	pH range	Number of samples	Toxic samples	Toxicity	Type of toxin		
					A	B	A & B
				pct.			
Cultivated soil	5.4 - 6.0	10	2	20.0	2	....	....
Cultivated soil	6.0 - 6.5	24	3	12.5	2	1	....
Cultivated soil	6.5 - 7.0	38	6	15.7	4	1	1
Cultivated soil	7.0 - 7.5	84	11	13.0	10	1	....
Cultivated soil	7.5 - 8.0	20	4	20.0	3	1	....
Cultivated soil	Over 8.0	2	....	.....	....	....	....
Virgin soil, coniferous	4.5 - 7.7	5	....	.....	....	....	....
Virgin soil, deciduous	4.38-5.95	17	1	5.8	1	....	....
Virgin soil, mixed	4.25- 6.9	38	2	5.3	2	....	....
Dusts	5.5 - 8.6	45	4	8.8	2	1	1

cent, type B toxin; and two, or 6.6 per cent, mixed type A and B toxin. Of the 178 cultivated soils 26, or 14.6 per cent, were toxic; three, or five per cent, of the 60 virgin soils and four, or 8.8 per cent, of the 45 samples of barn dusts were toxic. The distribution of the toxicity is summarized (Table 2).

The types of cultivated soils collected included muck, clay, sandy loam, and silt loams; the proportion of each soil collected and the relative toxicity is tabulated (Table 3). The series of silt loams collected included Honeoye, Amboy, Lyons, Dunkirk, Collamer, Camillus, Scholharie, Farmington, Chenango, Worth, Volusia, Wooster, Cazenovia, and Chippewa. Sandy loams belonged to the series of Dunkirk, Worth, and Miami, and clay soils were of the Fulton series. No correlation could be shown between toxicity and soil series.

TABLE 5

Toxicity of Soils in Relation to Crop

Crop	Number of samples	Toxic samples	Toxicity	Type of toxin		
				A	B	A & B
			pct.			
Corn	81	12	14.8	9	3	....
Beans	26	6	23.1	6	....	....
Unsown	40	5	12.5	4	1	....
Corn and beans	2	2	100.0	1	....	1
Potatoes	8	1	12.5	1	....	....
Other crops	21	....	.....	....	....	....
Total	178	26	14.6	21	4	1

Samples of virgin soil were obtained from 60 areas of forest land. Seventeen of these areas were covered with deciduous trees; five were mainly conifers; and 38 bore a mixed growth of conifers and deciduous trees. Three, or five per cent, of these samples yielded toxic cultures. All were type A; two of these came from soils bearing mixed vegetation and one from a deciduous forest.

The hydrogen-ion concentration of all samples of soils and dust collected ranged from 4.25 to 8.5. The toxic samples also showed wide variation in pH values, 4.25 to 8.

The crops growing on the cultivated soils included 81 of corn, 26 of string beans, eight of potatoes, five of oats, five of buckwheat, four of cabbage, three of alfalfa, two of mixed corn and beans, and one each of squash, carrots, wheat, and peas. Forty fields were unsown at the time of the collection of the soil. The fields sown to corn or beans or both yielded a higher percentage of toxic soils than those planted with other crops; the occurrence of toxic cultures in relation to the crop is summarized (Table 5).

DISCUSSION

It seems apparent from the results presented here that *Clostridium botulinum* is widely distributed within the central area of New York state. Though *Clostridium botulinum* type A was predominant, type B is also found both alone and in combination with type A. No other strains of

the organism were found. The predominance of type A is in agreement with the results of Dr. Meyer, who examined 27 soils of New York and found six of these to be toxic. Five of the toxic samples were type A, the remaining one being only weakly toxic and therefore not typed. The low percentage of toxic cultures demonstrated in virgin soils, however, fails to agree with the finding of Meyer, who has found such a large proportion of virgin soils to be toxic in contrast to cultivated soils that he has theorized that the organism is a natural inhabitant of virgin soils and from these is carried by the wind or other means to the valleys and foothills below mountain ranges. His results in contrast to those found in this study are presented (Table 6). This discrepancy in results at the present time can-

TABLE 6

Comparison of Results

Investigator	Locality	Virgin soils—toxicity	Cultivated soils—toxicity
		pct.	pct.
Meyer and Dubovsky (1922a)	California	57.6	26.9
Meyer and Dubovsky (1922b)	U. S. A.	24.7	12.4
This study	New York	5.0	14.6

not be fully explained. It does, however, indicate the necessity for further work involving determination of water content of soils, analyses of soils for other bacteria growing concomitantly with *Clostridium botulinum*, analysis of the chemical and organic constituents of various types of soil, collection of the history of crop cultivation and fertilization of soils, and other factors which have a bearing on the development of a specific bacillus in soil media.

That *Clostridium botulinum* may exist in the soil over a wide range of hydrogen-ion concentration is evident from its demonstration in soil of pH 4.25 to 8. This is surprising in view of *in vitro* experiments which have shown that a neutral to faintly alkaline medium has been best for growth and toxin formation of the organism. In addition, the majority of soil microorganisms in general do not tolerate a wide range of hydrogen-ion concentration. However, it is not so surprising in view of the bacteriological examination of food products which has shown that *Clostridium botulinum* will exist under a very wide pH range, especially when other bacteria, yeasts, and molds develop simultaneously as would be expected in the soil. The existence of *Clostridium botulinum* in the soils of a wide range of pH, however, is of significance in that certain crops are associated with soil of a definite range of pH so that it seems apparent that there would be no reason to assume that any one crop, in relation to the acidity or alkalinity of its substrate, would be safe from contamination from *Clostridium botulinum*.

The fact that the majority of toxic cultures in this study were associated with corn and bean crops is of interest in that these foods have been the cause of the greatest number of cases of botulism in this country. There are of course other factors to be considered in this respect but the

association may be of significance. It has been suggested that the preceding crop may be of some influence on the development of *Clostridium botulinum* in any given soil. The ploughing under of the remnants of the previous year's crop will influence the type of organic material in the soil. It has been found that certain types of bacteria have been associated with specific types of organic matter and some of these may have an inhibitory influence on *Clostridium botulinum* while others may stimulate its growth.

SUMMARY

This study comprises a bacteriological analysis for *Clostridium botulinum* of 283 samples of soil and dust collected in the five central counties of New York state. The soil specimens were obtained from cultivated and virgin areas. The dust was secured from barns and other farm buildings.

Clostridium botulinum, types A and B, was found to be distributed widely in this central area. Thirty-three, or 11.7 per cent, of the total samples collected yielded toxic cultures of *Clostridium botulinum.* Twenty-six, or 78.7 per cent, of these were type A; five, or 15.1 per cent, type B; and two, or 6.6 per cent, yielded a mixed toxin of types A and B.

The greatest percentage of toxic cultures has been obtained from soil of cultivated fields. Of the 178 samples obtained from this source 26, or 14.6 per cent, were toxic. Twenty-one, or 80 per cent, of these contained type A toxin; four, or 15.4 per cent, type B toxin; and one was a mixed type A and B culture. The greatest percentage of toxic cultivated soils was obtained from ground bearing crops of string beans. By far the greatest number of cultivated soils tested were silt loams as this soil predominates in these five counties. All of the toxic soils were silt loams.

Three, or five per cent, of the 60 virgin soils yielded toxic cultures, all of which were type A. Two of these soils were obtained from forests of mixed coniferous and deciduous growth and one from an area of deciduous trees only. No toxic cultures were obtained from coniferous forest land.

Four, or 8.8 per cent, of the 45 barn-dust samples yielded toxic cultures, two being type A, one type B, and the remaining one types A and B in a mixed culture.

The hydrogen-ion concentration of all soils was 4.25 to 8.5. Toxic cultures were obtained from soils in the range of pH 4.25 to 8.

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RELATIONSHIPS BETWEEN FRACTIONS OF PHOSPHORUS IN SOIL AND GROWTH OF CEREALS[1]

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The chemical properties of a group of soils in the vicinity of Midland, Douglas County, Kansas, have been described in detail by Wynd and Romig (1943). Wynd and Noggle (1943) later reported that the amount of chemosorbed phosphorus was related directly to the amount of organic matter and nitrogen in the soil. The phosphorus fractions, designated as P_1 and chemosorbed plus easily-acid-soluble, were also found to be more or less related in amount to the organic matter by virtue of the chemosorbed phosphorus which these fractions include. There was usually also a positive relationship between each of these phosphorus fractions and the amounts of replaceable bases because the amount of organic matter in these soils determines to a very great extent their base-exchange capacity. The base-exchange capacity, in turn, frequently governs the amount of replaceable bases.

The amounts of easily-acid-soluble phosphorus, unlike chemosorbed phosphorus, showed no relationship with the organic matter present nor with any property of the soil which was related to the organic matter.

The observations summarized above suggest that any fraction of soil phosphorus composed largely of chemosorbed phosphorus would exhibit definite correlations with the growth of plants, while fractions composed largely of easily-acid-soluble phosphorus would show such correlations only under unusual and extreme circumstances. The great importance of defining as accurately as possible the relationships between properties of soil and the growth of plants encouraged the authors to study the associations which exist between each of the four fractions of soil phosphorus described above and the growth of cereals.

MATERIALS AND METHODS

A group of 15 fields in the vicinity of Midland, Douglas County, Kansas, were selected for study. Oats were grown on seven of these soils during the autumn of 1940, and rye was grown on eight of these fields during the spring of 1941. The crops were harvested just as jointing occurred, and the material was dried immediately in an Arnold hot-air dehydrator. The entire yield from each field was used to calculate the yield per acre; the data are presented (Table 1). The file numbers refer

[1] The expenses incurred during this study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

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to the original samples preserved in the Department of Botany and Plant Pathology at Michigan State College. The point numbers enable the reader to identify the soils in the table from which the data in the figures were obtained.

The details of the chemical properties of these soils may be determined by referring to corresponding file numbers in the tables published by Wynd and Romig (1943). The methods by which the phosphorus fractions were determined are described by Wynd and Noggle (1943). Statements concerning the amounts of replaceable bases in these soils are based on the data presented in the tables which appeared in these publications.

TABLE 1

Phosphorus Fractions in Soil and Growth of Cereals[1]

File No.		Field No.	Point No.	Date harvested	Phosphorus fractions				Yield of dry matter per acre
Soil	Crop				P_1	Chemo-sorbed	Chemo-sorbed plus easily-acid-soluble	Easily-acid-soluble	
				1940	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*lb.*
267	315	2A	1	Oct. 8	105	118	176	58	221
268	317	2B	2	Oct. 11	104	90	168	78	304
269	319	19	3	Oct. 17	58	60	110	50	200
270	320	11E	4	Oct. 18	76	63	95	32	200
272	321	12	5	Oct. 21	165	113	170	57	389
274	318	14B	6	Oct. 23	67	60	138	78	253
280	325	18	7	Nov. 1	100	93	160	67	552
				1941					
286	332	9	1	Apr. 10	60	38	107	69	144
285	334	10	2	Apr. 11	56	45	128	83	221
288	335	20	3	Apr. 12	70	50	112	62	379
284	336	19B	4	Apr. 13	45	38	88	50	500
287	337	22	5	Apr. 14	51	35	98	63	298
291	338	11	6	Apr. 17	74	63	134	71	947
289	339	15A	7	Apr. 20	100	78	170	92	1430
290	340	15B	8	Apr. 20	104	88	197	109	1950

[1] The first group of points, 1 to 7 inclusive, refers to oats, and the second group of points, 1 to 8 inclusive, refers to rye.

A comparison of the productivity of soils must be based arbitrarily upon some accurately defined response of a specified crop. This arbitrary selection of the standard for comparison makes possible various, and perhaps contradictory, definitions of fertility. In the present study the dry weight of the crop, harvested just as jointing occurred, was selected as the basis for comparison because the tissue of cereal plants contains especially high concentrations of protein, vitamins, and minerals at this stage of physiological development. The extensive use of cereal plants as sources of protein and vitamins for use as human food and for stock consumption, makes the study of the nutritional conditions which influence the yield of dry matter at this stage of especial importance.

EXPERIMENTAL RESULTS

P_1 Phosphorus Fraction: The data presented (Figs. 1 and 2) show that the greater growth of both oats and rye occurred in soils containing the

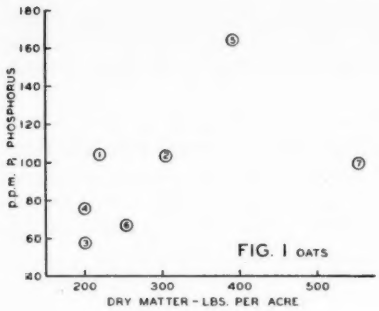

FIG. 1 OATS

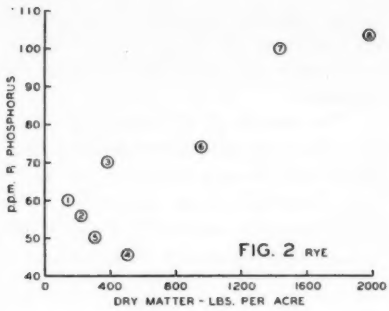

FIG. 2 RYE

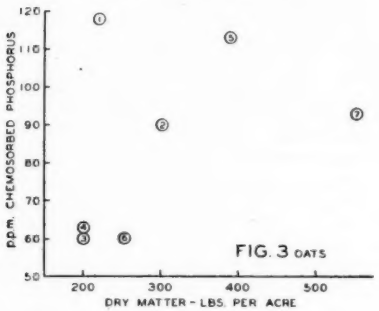

FIG. 3 OATS

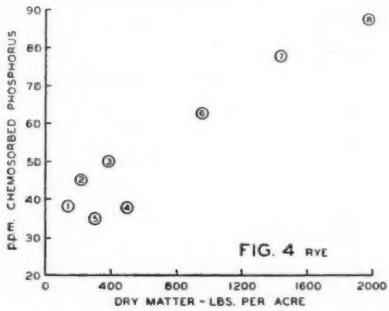

FIG. 4 RYE

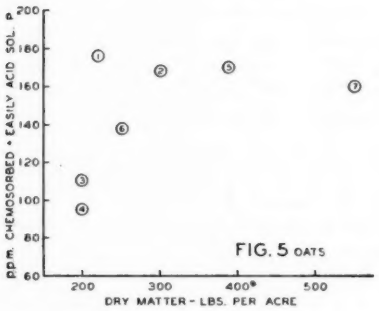

FIG. 5 OATS

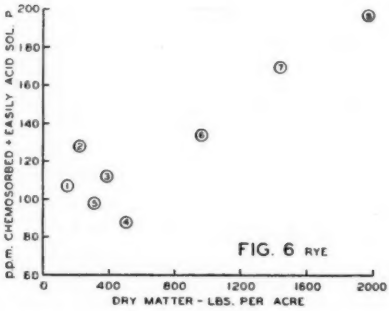

FIG. 6 RYE

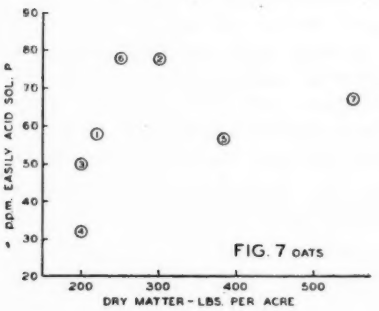

FIG. 7 OATS

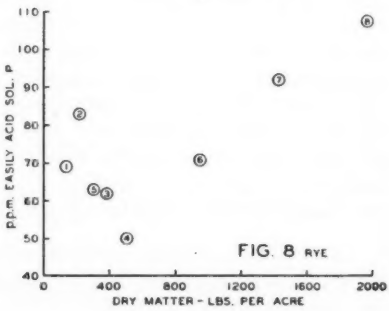

FIG. 8 RYE

greater amounts of phosphorus in the P_1 fraction. Point 7 (Fig. 1) represents a soil that is exceptionally rich in bases, and it appears that the growth of oats in this soil was influenced more by this circumstance than by the moderate amount of phosphorus present in the P_1 fraction.

Chemosorbed Phosphorus Fraction: Figures 3 and 4 show that the amount of chemosorbed phosphorus in the soil is closely related to the growth of oats and rye. Point 1 (Fig. 3) represents data obtained from a soil which contained a comparatively small amount of replaceable bases, while Point 7 represents a soil exceptionally rich in bases. This suggests that the amount of replaceable bases was more important than the amount of chemosorbed phosphorus in determining the growth of oats on these soils.

Chemosorbed Plus Easily-Acid-Soluble Phosphorus Fraction: Figures 5 and 6 indicate a general relationship between the amount of chemosorbed plus easily-acid-soluble phosphorus in the soil and the growth of oats and rye. The amounts of phosphorus in this fraction are about equal in the soils represented by Points 1, 2, 5, and 7 (Fig. 5) even though large differences in growth occurred on these soils. The data of Wynd and Romig (1943) show that the soils comprising this group contain replaceable bases in amounts which are directly proportional to the growth of oats. This indicates that the amount of replaceable bases in these soils is more important than the amount of chemosorbed plus easily-acid-soluble phosphorus in governing the growth of cereals.

Easily-Acid-Soluble Phosphorus Fraction: Figures 7 and 8 show that there is no well-defined relationship between the amount of easily-acid-soluble phosphorus in the soil and the growth of oats and rye. Comparison of the data (Fig. 7) with additional chemical data of Wynd and Romig (1943) obtained from these soils shows that the growth of oats parallels very closely the amount of replaceable bases, which suggests that the amounts of easily-acid-soluble phosphorus are of secondary importance. On the other hand, the data for the growth of rye (Fig. 8) are more difficult to interpret. Points 1 to 5, inclusive, indicate less growth is obtained when larger amounts of easily-acid-soluble phosphorus are present, but Points 4, 6, 7, and 8 indicate that this interpretation is not necessarily true. It is evident that some factor, or factors, other than easily-acid-soluble phosphorus is governing the growth of rye on these fields.

DISCUSSION

A study of the data presented in Table 1 and in the figures shows that the amount of growth of oats and rye is positively related to the amounts of phosphorus in the soil present in fractions designated as P_1, chemosorbed, and chemosorbed plus easily-acid-soluble. However, the growth of these cereals does not seem to be related to the amount of phosphorus present in the easily-acid-soluble form.

The P_1, chemosorbed, and chemosorbed plus easily-acid-soluble fractions are composed largely of chemosorbed phosphorus. Wynd and Romig (1943) have pointed out that the amounts of these fractions are related directly to the organic matter, nitrogen, and the base-exchange capacity of the soils, mostly because of the relation which exists between chemosorbed

phosphorus and organic matter. The base-exchange capacity determines the amount of replaceable bases retained in many of these soils. A critical study of the figures in the present paper and of the tables of additional chemical data of Wynd and Romig (1943) discloses that the amounts of growth of oats and rye are correlated with these three phosphorus fractions *only* if the amounts of replaceable bases vary simultaneously and in the same direction as the amounts of phosphorus. Usually this situation does exist, but in a few instances it does not. In these exceptional instances the amounts of replaceable bases govern the growth, irrespective of the amounts of phosphorus present.

The amounts of easily-acid-soluble phosphorus do not appear to be related consistently to the growth of oats and rye. In the authors' previous paper (1943) it was stated that this fraction of soil phosphorus was not related to the base-exchange capacity, organic matter, nitrogen, and the replaceable bases. Since it is apparent from the discussion above that the amounts of replaceable bases are of primary importance in governing the growth of oats and rye, correlation between this phosphorus fraction and growth could not be expected. The degrees of association between the growth of cereals and the magnitudes of this phosphorus fraction (Figs. 7 and 8) probably depend on the amounts of replaceable bases rather than on the phosphorus.

It is apparent that any attempt to interpret the basis of soil productivity by the study of a limited group of factors is almost certain to lead to false conclusions. Properties of the soil are so intimately related to each other that large masses of data concerning many factors must be accumulated before fundamental relationships to plant nutrition become apparent. The data presented in the present paper were obtained from soils in the vicinity of Midland, Douglas County, Kansas, and generalizations concerning other areas must be avoided until more data on different soils have been accumulated.

SUMMARY

The amounts of phosphorus present in four fractions designated as P_1, chemosorbed, chemosorbed plus easily-acid-soluble, and easily-acid-soluble were determined in 15 soils in the vicinity of Midland, Douglas County, Kansas.

The yield of dry matter of oats and rye, harvested at the jointing stage, was determined from each of these soils.

Phosphorus is not a factor limiting the growth of cereals in the soils studied.

The amount of replaceable bases appears to be the primary chemical factor limiting the growth of cereals on the soils studied.

The growth of oats and rye was positively related, in most instances, to the amount of phosphorus present in the P_1, chemosorbed, and chemosorbed plus easily-acid-soluble fractions, but *only* if these amounts of phosphorus were positively related to the amounts of replaceable bases.

The amounts of easily-acid-soluble phosphorus exhibited a poorly defined relationship to the growth of oats and rye.

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FACTORS AFFECTING SOME PHYSICOCHEMICAL PROPERTIES OF STARCH[1]

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A number of papers have been published dealing with the viscosity, swelling power, and gel strength of starch. These papers will not be summarized in the present report as it has been satisfactorily done by authors whose work is included in the references. Only studies directly related to this investigation will be referred to, and for more detailed information the interested reader should consult the papers noted.

Ripperton (1931) described a series of experiments conducted on canna and potato starches in which swelling power and viscosity were determined. Decided differences in these properties were found among the starches. The addition of electrolyte at time of cooking the starch had a marked influence on swelling power and viscosity, owing apparently to the positive electric charge carried by the cation, since the degree of the effect varied with the cation valency and the anions were found to be without significant effect. In general, cation additions decreased swell and viscosity. Tanner and Englis (1940) reported little variation in swell among seven samples of starch prepared from different varieties of corn but obtained some evidence that size of granule may influence viscosity. The hard starches formed more viscous pastes than soft starches of the same granular size.

Woodruff and MacMasters (1938) discovered significant differences in the gel strength of starches from various varieties of corn as well as from corn grown under different environmental conditions. Viscosity variations were very small compared with differences in gel strength. Knowles and Harris (1943) examined the gel strength of starches prepared from different wheat sorts, as well as from wheats harvested at different stages of maturity, and concluded that both wheat variety and maturity influenced gel strength. Environmental conditions during growth of the wheat plant also affected gel strength. Harris, Sibbitt, and Toman (1944), working with a series of hard red spring wheat varieties grown at four North Dakota stations in 1942, secured confirmatory evidence respecting varietal and environmental influences on gel strength.

The following investigation was undertaken to ascertain the effect of wheat maturity upon certain physicochemical properties of starch, and to elucidate the influence of treatment by cooking with electrolytes containing ions of different valencies on the swelling power and viscosity of starch. In addition the effect of the "amylodextrin"[2] component on starch prop-

[1] Published with approval of the director of the North Dakota Agricultural Experiment Station.

[2] The term "amylodextrin" as used in this paper denotes the "small-granule" or "tailings fraction" studied by MacMasters and Hilbert (1944). These latter terms appear to be more suitable since true amylodextrin is a recognized degradation product of starch.

erties was observed. The portion of this work concerned with gel strength is an extension of similar experiments reported by Knowles and Harris (1943).

EXPERIMENTAL PROCEDURE

Three of the wheat starches employed in this investigation were prepared from commercially milled, hard red spring wheat flours. The remainder were from experimentally milled, long-patent flours of approximately 70-per cent extraction. The wheats were grown in North Dakota, Kansas, Illinois, and Indiana and consisted of samples of hard red spring, hard red winter, soft red winter, and durum. The method of taking the samples in the maturity studies varied somewhat among the different series, but in general the heads were immediately removed from the straw to avoid transfer of material to the head. In some instances, the heads were air-dried; in others, the more immature samples had the kernels removed from the heads the day the samples were secured, and the grain was then air-dried under a fan. No doubt the percentage composition of the kernels was changed by respiration and synthesis during drying, as found by McCalla and Newton (1935), but it was necessary to dry the grain before milling.

The barley and rice starches were separated from experimentally milled barley and rice flours, respectively, two barleys of different malting quality being used, while the rice was from bulk unpolished grain purchased in a grocery store. The potato starch, used to secure a comparison between the cereal starches and a root starch, was prepared from potatoes grown at the Experiment Station at Fargo, North Dakota.

The wheat starches, with one exception, were prepared essentially by the procedure described by Harris and Sibbitt (1941). The soft red winter wheat starch was prepared by the method described by Woodruff and MacMasters (1938) and was three years old. It was found necessary to treat the barley and rice flours with 0.5 per cent NaOH to extract the starch from the protein matrix. One hundred and fifty grams of flour was thoroughly stirred with 500 c.c. of the alkali and allowed to stand 18 to 20 hours at 5°C.(41°F.) with occasional stirring. No gelatinization was observed under these conditions. The starch mixture was centrifuged and the supernatant liquid decanted, thus removing the alkali solution. The starch was then thoroughly washed with distilled water, followed by redistilled water, and dried by fanning. It was observed that the barley starches required more centrifugation to obtain a satisfactory separation than the rice or potato starches.

The root starch was prepared by thoroughly washing a suitable quantity of fresh sound potatoes with tap water, peeling the potatoes and slicing them into thin strips which were then disintegrated for five minutes in a Waring blendor with distilled water. The resultant sludge was transferred to coarse bolting cloth and the liquid containing most of the starch was worked through the cloth. The pulp remaining on the cloth was washed twice with distilled water and discarded. The liquid was strained through a second finer cloth and the suspended starch allowed to settle. The starch was recovered from the liquid suspension by decantation and centrifuging.

It was washed a number of times, first with tap water and then with distilled water, before drying.

Moisture and nitrogen determinations were made according to "Cereal Laboratory Methods," fourth edition (1941). The pH determinations were based on the method of Ripperton (1931) in which five grams of starch was suspended in 100 c.c. of redistilled water with constant, low-speed stirring. It was found that a 15-minute period gave consistent results. A Beckmann pH meter was employed with a glass electrode. Gel strength was ascertained by the method described by Knowles and Harris (1943) and Harris, Sibbitt, and Toman (1943). The viscosity was found with the Hoeppler viscosimeter employing one-per cent (dry-matter basis) suspen-

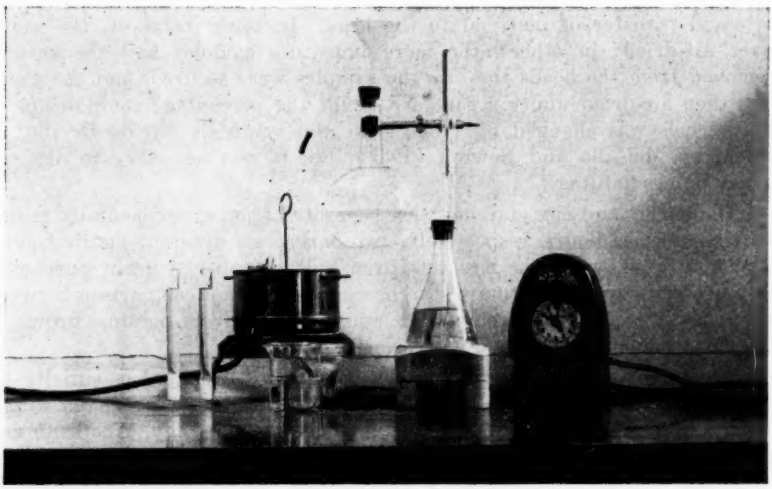

FIG. 1. Equipment used in determining the swelling power of starch.

sions gelatinized at 95°C.(203°F.) and cooled to 25°C.(77°F.) for several hours before being placed in the viscosimeter tube. Three determinations were made on each starch solution, and the procedure was repeated on the following day, using a fresh sample of starch. There was considerable disparity among the replicated results, particularly among triplicates from different gelatinizations, as found by Ripperton.

Swelling power was secured by a modified form of the method of Ripperton (1931) in which 0.1 gram (dry-matter basis) of starch was treated with 10 c.c. of redistilled water at 80°C.(176°F.) in 50-mm. Pyrex test tubes 12 mm. in diameter.

The equipment utilized in making the swelling-power determinations consisted primarily of a copper bath fitted with a double-shelf, test-tube support, capable of holding a number of 12-mm. test tubes, and an electric hot plate (Fig. 1). The foot of the tubes did not touch the bottom of the bath, so local overheating was prevented. A second electric plate equipped with a three-heat switch served to maintain the redistilled water used in

cooking the starch at 80°C. The water was contained in a 700-c.c. Erlenmeyer flask fitted with a double-hole, tubulated stopper to accommodate a thermometer and 10-c.c. pipette. The pipette was allowed to remain in the flask when not actually in use. Tap water at room temperature was siphoned from a one-liter Erlenmeyer flask at a rate just sufficient to replace the water evaporated from the bath. The water was kept approximately one centimeter above the surface of the cooking liquid in the test tubes. The addition of the water had no cooling effect on the temperature of the bath water, which was kept boiling vigorously. The test tubes were not covered during the cooking period.

After the starch and water were thoroughly mixed, the tubes were immediately placed in a boiling-water bath for 15 minutes. The tubes were then removed from the bath and the contents stirred gently. The starch solution was transferred to 25 c.c. graduated cylinders,[3] and the test tubes carefully rinsed several times with boiled, redistilled water. The rinsing were added to the cylinders which were filled to the mark, inverted several times to thoroughly mix the contents, and allowed to stand overnight. The volume of the swollen starch granules was read the following morning, to the nearest 0.1 c.c., using a fluorescent lamp and reading glass. Swelling power is defined as the volume in cubic centimeters of the swollen granules of one gram of dry starch cooked by the procedure outlined. The electrolytes were added in 0.01 N solution to the starch, and the volume of the solution subtracted from the 10 c.c. of redistilled water employed in cooking. Unless otherwise noted, all swelling-power results are the average of eight replications.

DISCUSSION OF RESULTS

Effect of Wheat Maturity: The studies concerned with the effect of maturity on various starch properties fall into two series; the first comprises one hard red spring and one durum wheat grown at Fargo, North Dakota. The former was grown in both 1941 and 1942, but the durum was grown only in 1942. In addition a second variety of hard red spring wheat and one soft red winter wheat, Thorne, were grown at Peoria, Illinois, in 1942. The second series embraces four samples each of hard red spring and durum wheat grown at Fargo in 1943. The 1941 Thatcher starch gel strength was reported by Knowles and Harris (1943), but it is included in the present study to afford a comparison with the gel strength of the other samples. The data obtained from the more extensive first series are shown (Table 1), while data from the second series are presented (Table 2). Since sufficient starch was not available in many of the samples of the first series for the determination of gel strength and viscosity, the swelling power alone was ascertained. Some pertinent data secured from the wheats are included in each table to permit estimation of the relationship between wheat maturity and starch properties.

It is quite apparent that starch gel strength increases with maturity in all wheat sorts, as reported by Knowles and Harris (1943). There is also evidence that starch viscosity tends to increase as the wheat ripens.

[3] For untreated potato starch 50- or 100-c.c. graduated cylinders were used and filled to the mark.

Swelling power, however, decreases with maturity for all wheats examined. Barham, Wagoner, Williams, and Reed (1944) found that the viscosity of starch from late-harvested potatoes was lower than for similar potatoes harvested early. Corresponding to these changes in the physicochemical properties of the starch there are very marked increases in the number of vitreous wheat kernels and test weight per bushel, while wheat moisture content decreases greatly (Tables 1 and 2). There is some evidence of an increase in wheat protein content with maturity, but it is not very consistent. It is difficult to postulate significant differences in starch properties between the various wheats since the samples are not strictly comparable. There seems to be some evidence that the durum starch is slightly higher in gel strength and viscosity, but no marked differences are apparent in swelling power between the different varieties at Fargo; however, the wheats grown at Peoria were significantly higher in swelling power.

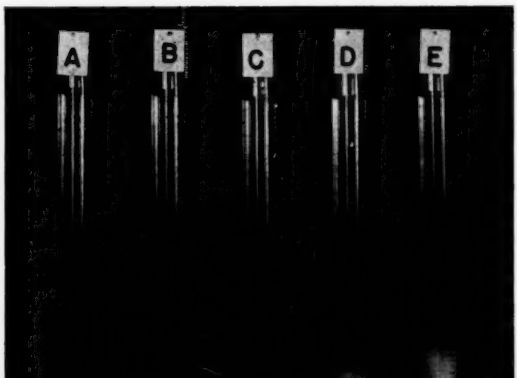

FIG. 2. Comparative volume of swollen starch following treatment with electrolytes of varying cation valency. A is the control, with no electrolyte added. B, C, D, and E were each treated with electrolytes at 0.001 N concentration in the following order: NaCl, $MgSO_4$, $Al_2(SO_4)_3$, and $Th(NO_3)_4$.

Effect of "Amylodextrin": The influence of the "amylodextrin" fraction on some starch properties was quite significant (Table 3). The nitrogen content was higher in starches with the "amylodextrin" for each wheat in agreement with the findings of MacMasters and Hilbert (1944), who reported four to eight times more nitrogen in "amylodextrin" than in starch itself. The pH values were also lower in the starches containing this fraction. Gel strength and swelling power were lowered by "amylodextrin" but viscosity was raised, and this is what one would expect from *a priori* considerations since this fraction has been shown by MacMasters and Hilbert to consist largely of very small and damaged starch granules. Knowles and Harris (1943) also found that gel strength was decreased by "amylodextrin." No effort was made to examine this fraction separately

TABLE 1

Effect of Maturity on Starch Swelling Power and Other Properties of Wheat

Wheat variety [1]	Date of harvest	State of maturity	Approximate vitreous kernels	Test weight	Wheat moisture at harvest	Wheat protein	Starch nitrogen content	Swelling power [2]
			pct.	*lb./bu.*	*pct.*	*pct.*	*pct.*	*c.c.*
GROWN AT FARGO—1941								
Thatcher H.R.S.	July 19	Green	20	39.9	.....	13.2	.11	31.1
Thatcher H.R.S.	July 24	Green	60	51.7	.....	13.1	.12	28.7
Thatcher H.R.S.	July 28	Slightly green	75	57.2	.....	13.9	.11	27.4
Thatcher H.R.S.	Sept. 3	Heavily bleached	95	54.4	.....	14.8	.12	26.9
						13.7[3]	....	28.5[3]
GROWN AT FARGO—1942								
Thatcher H.R.S.	July 20	Green	65	52.6	63.8	14.9	.13	29.5
Thatcher H.R.S.	July 23	Green	75	57.9	54.3	15.1	.12	29.2
Thatcher H.R.S.	July 27	Green	85	61.4	49.4	15.4	.11	27.9
Thatcher H.R.S.	July 30	Slightly green	95	62.6	40.8	15.5	.10	27.8
Thatcher H.R.S.	Aug. 3	Green	95	60.6	22.7	15.2	.10	26.5
Thatcher H.R.S.	Aug. 6	Ripe	95	60.1	22.9	14.8	.08	27.6
						15.1[3]	....	28.1[3]
Durum	July 24	Green	10	45.5	72.1	13.5	.17	31.1
Durum	July 28	Green	30	54.4	66.5	12.4	.13	30.7
Durum	July 31	Slightly green	65	61.6	57.1	12.5	.10	29.3
Durum	Aug. 4	Slightly green	75	64.5	51.2	12.6	.09	27.6
Durum	Aug. 7	Slightly green	85	65.3	52.3	12.9	.08	26.7
Durum	Aug. 11	Ripe	95	65.1	40.4	13.2	.07	26.0
Durum	Aug. 15	Ripe	90	65.3	28.4	13.4	.07	27.2
						12.9[3]	....	28.4[3]
GROWN AT PEORIA—1942								
Thorne S.R.W.	16[4]	Slightly green	....	55.3	.....	8.6	.09	34.4
Thorne S.R.W.	20	Slightly green	....	59.2	.....	9.8	.07	35.3
Thorne S.R.W.	23	Ripe	....	59.7	.....	10.0	.07	33.9
						9.5[3]	....	34.5[3]
Pilot H.R.S.	20	Green	18	45.9	.....	12.7	.06	32.6
Pilot H.R.S.	28	Slightly green	80	54.5	.....	13.9	.08	29.5
Pilot H.R.S.	35	Ripe	90	55.1	.....	14.9	.06	29.7
						13.8[3]	....	30.6[3]

[1] H.R.S. means hard red spring wheat; S.R.W. means soft red winter wheat. [2] Swelling power is the volume (c.c.) of swollen granules of one gram of dry starch cooked by the procedure used. [3] Varietal average. [4] The remaining figures in this column indicate days from pollination.

TABLE 2

Effect of Maturity on Some Wheat and Starch Properties

Type of wheat	Date of harvest	State of maturity	Approximate vitreous kernels	Test weight	Wheat moisture	Wheat protein	Starch nitrogen content	Gel strength[1]	Viscosity[2]	Swelling power[3]
			pct.	*lb./bu.*	*pct.*	*pct.*	*pct.*	*sec.*	*t./tw.*	*c.c.*
Hard red spring	July 16	Green	15	42.8	69.0	14.3	.13	26.0	1.37	32.1
Hard red spring	July 20	Green	65	54.3	59.8	13.6	.11	32.8	1.38	31.4
Hard red spring	July 27	Ripe	95	60.9	47.7	13.4	.09	34.0	1.58	27.3
Hard red spring	Aug. 2	Ripe	95	62.1	24.7	14.3	.12	35.3	1.45	27.1
				55.0[4]	50.3[4]	13.9[4]	....	32.0[4]	1.44[4]	29.5[4]
Durum	July 22	Green	20	46.0	69.0	12.8	.13	28.7	1.50	31.9
Durum	July 28	Slightly green	65	60.1	57.4	12.3	.08	32.4	1.60	25.9
Durum	Aug. 3	Ripe	85	61.2	51.2	12.2	.07	39.2	1.61	29.0
Durum	Aug. 10	Ripe	90	62.3	24.4	13.7	.08	39.1	1.65	27.6
				57.4[4]	50.5[4]	12.7[4]	....	34.8[4]	1.59[4]	28.6[4]

[1] Gel strength is recorded in number of seconds required to break the surface of the starch gel. [2] Viscosity is time of fall of ball in one-per cent starch solution divided by time of fall in redistilled water. [3] Swelling power is the volume (c.c.) of swollen granules of one gram of dry starch cooked by the procedure used. [4] Varietal average.

since it was difficult to dry and reduce the starch granules to satisfactory fineness. After drying, Mida starch exhibited the greatest difference in gel strength between the starches with and without "amylodextrin," but it is impossible to state at this time whether this difference is significantly greater than that between similar starches prepared from Vesta, the other hard red spring wheat included in this study.

TABLE 3

Effect of Wheat Variety and "Amylodextrin" Fraction on Some Starch Properties

Wheat variety [1]	Flour protein	Starch nitrogen content	Starch pH	Gel strength	Viscosity	Swelling power
	pct.	*pct.*		*sec.*	*t./tw.*	*c.c.*
Mida	14.0	.08	5.4	30.0	1.64	25.5
Mida	14.0	.04	6.2	42.1	1.59	28.3
Vesta	13.3	.07	5.9	33.9	1.51	27.2
Vesta	13.3	.04	6.3	37.7	1.49	29.9
Durum	12.0	.08	6.0	33.8	1.73	24.1
Durum	12.0	.04	6.5	33.7	1.63	27.2

[1] First row of starch data for each variety refers to starch with the "amylodextrin" fraction included, while the second row refers to starch with this fraction removed.

Starches From Different Cereals: A description of the source of starches employed in determining the effect of nature of the electrolyte on starch swelling power with comparative starch nitrogen and moisture content, and the viscosity data are shown (Table 4). The nitrogen content of several starches is remarkably low, and in the instance of the rice and barley starches this may likely be ascribed to the NaOH treatment employed in separating the starch from the flour. The moisture content is more variable, but all drying was done at the same temperature [34°C.(93.2°F.)] under vigorous air circulation. There are relatively enormous differences in viscosity between the potato and the cereal starches, especially for untreated starch. The differences among the untreated cereal starches are not great, but some appear to be significant. Rice starch had the highest viscosity, and the soft red winter wheat starch had the lowest. The latter may have been affected by mode of preparation and age of the starch. There is little evidence to support the contention that durum and hard red winter wheat starches have higher viscosities than hard red spring wheat starch. The chief point of interest in these data lies, however, in the effect of electrolyte treatments at cooking. The viscosity of the potato starch is greatly reduced by these additions, the effect increasing with cation valence, as found by Ripperton. The effect on the cereal starches is much less; in fact the net result of the NaCl treatments is to reduce the viscosity but slightly below the untreated value. The $MgSO_4$ additions apparently raise the viscosity over the untreated results for every cereal starch. Preliminary experiments with varying concentrations of electrolyte showed a gradual reduction in potato-starch viscosity, but little effect on cereal starches, owing in large part to the comparatively low viscosity of these latter starches.

The authors realize that single-point determinations of starch viscosity are limited in their reliability. Viscosity will vary with the previous his-

tory of the starch paste, and the complete picture can be secured only from a series of curves. Under some conditions the curves for one starch will intersect those for another starch, and therefore, the viscosity relations of the two starches might be completely reversed, depending upon the points chosen for comparison. The MacMichael or Stormer types of viscosimeters are apparently better adapted to the determination of the viscosity of starch pastes than the Hoeppler and Ostwald instruments.[4]

TABLE 4

Comparative Data Secured From Different Starches[1]

Source of starch	Nitrogen content	Moisture content	Viscosity as affected by addition of electrolytes[2]		
			Untreated	NaCl	$MgSO_4$
	pct.	*pct.*	*t./tw.*	*t./tw.*	*t./tw.*
Potato	.02	14.0	1042.91	12.36	7.02
Rice	.01	11.1	2.50	2.20	2.98
Barley (Trebi)	.03	10.0	1.96	1.89	4.02
Soft red winter wheat	.06	13.3	1.53	1.63	2.06
Hard red spring wheat	.05	13.7	2.01	2.09	2.77
1943 crop					
Durum wheat	.07	10.9	1.98	1.61	2.32
Hard red spring wheat	.05	16.0	2.13	1.90	2.65
1944 crop					
Barley (Wisconsin 38)	.03	10.0	2.02	1.89	2.90
Hard red winter wheat	.03	12.4	1.88	1.93	3.04
Average viscosity of cereal starches	....	.....	2.25	1.89	2.84

[1] Arranged in order of decreasing swelling power of untreated starch. [2] Electrolyte concentration in cooking solution = 0.001N, one-per cent (dry basis) starch solutions used.

Effect of Electrolytes on Swelling Power: The nine starches (Table 4) were employed in a series of swelling-power investigations with the primary purpose of ascertaining the effect on swell of different electrolyte treatments at cooking time. This procedure was used by Ripperton (1931) with canna and potato starches, and surprising reductions in swell were found with these root starches. Preliminary work showed that electrolytes reduced the swell of the potato starch, the reduction increasing as the concentration of electrolyte in the cooking liquid was increased, as described by Ripperton. The reduction was also increased at equivalent concentrations by replacing monovalent by divalent salts. With cereal starches, however, the effect was reversed and an increase in swell with augmented electrolyte concentration on valency was noted. A series of electrolyte additions was made at time of cooking to all nine starches, using four salts of increasing cation valency (Table 5), since it was suspected that the positive ion was responsible for the changes remarked when salts were added. Samec (1927) found that starch absorbed the positive ion significantly, resulting in a decrease in particle charge and swelling power. Ripperton has confirmed this effect in his swelling-power studies on root starches and has attributed it to the partial neutralization of the negative

[4] Private communication from Dr. M. M. MacMasters, Northern Regional Laboratory, Peoria, Illinois.

charge on the starch granule in colloidal solution, yielding a "quasi-voluminous" effect. If the particles are negatively charged, they surround themselves with a repellent sphere that increases the active volume. There is also probably some effect on water imbibition. As the electrokinetic potention is reduced the mutual attraction of the starch particles is decreased with the result that the effective size of the particle is lessened, with the net result that the starch particles move closer together, thus decreasing the swell.

TABLE 5

Effect of Valency of Cation on Swelling Power of Various Starches[1]

Source of starch	Control	Electrolyte treatment			
		NaCl	$MgSO_4$	$Al_2(SO_4)_3$	$Th(NO_3)_4$
	c. c.	c. c.	c. c.	c. c.	c. c.
Potato	450.0	210.0	128.9	80.6	130.1
Rice	40.7	42.5	38.0	54.8	61.6
Barley (Trebi)	33.6	30.2	31.2	42.9	63.7
Soft red winter	32.4	33.2	38.4	52.1	66.2
Hard red spring, 1943 crop	29.7	27.6	36.3	38.5	67.9
Durum	27.0	28.8	42.1	39.5	73.0
Hard red spring, 1944 crop	26.8	27.7	34.5	40.5	64.7
Barley (Wisconsin 38)	26.0	28.3	29.0	50.4	59.2
Hard red winter	25.8	29.9	38.7	42.4	59.9
Mean swelling power of cereal starches	30.3	31.0	36.0	45.1	64.5
Potato[2]	.....	—240.0	—321.1	—369.4	—319.9
Cereal[2]	.....	+0.7	+5.7	+14.8	+34.2

[1] Arranged in order of decreasing swelling power of untreated starch. [2] These figures show the changes in swelling power induced by added electrolyte.

The reversal of the trend toward increasing swelling power with electrolytes evidenced by the cereal starches might be explained by assuming that the negative electropotential on a cereal-starch particle is substantially lower than on a root starch, and it is accordingly neutralized rather quickly by positive cations. Sodium chloride has little influence on the swell of cereal starches, although it has a very marked effect on potato starch (Table 5). With $MgSO_4$ the increase in swell becomes undoubtedly significant and increases relatively more with $Al_2(SO_4)_3$ than with $Th(NO_3)_4$. The swell induced by the latter salt was twice that of the untreated starch. These results would seem to indicate that the electrokinetic potential of the cereal-starch particle has been reversed in sign and increased by the addition of cations with resultant raising of the hydration. The potato-starch swell, which had been reduced to approximately one-third of the control by $Al_2(SO_4)_3$, was significantly increased by $Th(NO_3)_4$. One might conclude from these results that the electrokinetic potential of the potato-starch granule had been reversed in sign and increased by the tetravalent thorium ion, with a concomitant increase in swelling power owing to increased electroviscous effects.

Although thorium is highly hydrated, the hydrogen ions contributed by the formation of HNO_3 would have little appreciable effect on swelling power, as Ripperton showed that the effect of hydrogen as a cation was of the same order of magnitude as monovalent ions. For potato starch, the

effect of 0.001 N acid should be to depress the swell approximately 240 c.c. Thorium nitrate, however, had less depressive action than $Al_2(SO_4)_3$, and this can best be explained by postulating a reversal of electrokinetic potential of the starch particle. In the instance of the cereal starches, it is quite obvious that no significant action can be exerted by hydrogen ions since the sodium ion increased the swelling only 0.7 c.c. while $Th(NO_3)_4$ increased it 34.2 c.c. There appears to be little consistent difference among the cereal starches in response to electrolyte treatments. No significant changes in gel strength were caused by electrolyte additions before gelatinizing the starch.

TABLE 6

Effect of Valency of Anion on Swelling Power of Cereal Starches

Source of starch	Control	Electrolyte treatment			
		NaCl	Na_2SO_4[1]	Sodium citrate[1]	$K_4Fe(CN)_6$[1]
	c.c.	c.c.	c.c.	c.c.	c.c.
Hard red spring	26.8	27.7	27.6	27.1	27.4
Barley (Wisconsin 38)	26.0	28.3	29.2	29.9	29.4

[1] Average of four determinations.

TABLE 7

Effect of Reduction of Cation on Swelling Power of Cereal Starches[1]

Source of starch	Electrolyte added at cooking	Washing liquid	Control	Number of washings		
				1	2	3
			c.c.	c.c.	c.c.	c.c.
Durum	$MgSO_4$	Na citrate	41.8	32.0	24.3	16.0
		Distilled water	43.4	34.0	25.5	21.5
Durum	$Th(NO_3)_4$	Na citrate	68.5	45.0	38.0	35.3
		Distilled water	61.0	51.5	45.5	42.0
Hard red spring	$MgSO_4$	Na citrate	31.0	26.5	25.5	22.9
		Distilled water	35.0	30.0	27.9	25.5
Hard red spring	$Th(NO_3)_4$	Na citrate	63.0	40.0	36.0	36.0
		Distilled water	63.0	57.0	48.0	40.0

[1] Average of four determinations.

Two further experiments were made to obtain additional insight into the action of ions on cereal-starch swelling power. In the first, four salts with anions increasing progressively in valence from one to four, were used at time of cooking in equivalent concentration to the salts already employed. As anticipated, no increase in swelling power was obtained, except a slight change that could be attributed to the positive ion (Table 6). This result is very different from the values obtained with salts with increasing cation valency (Table 5). The second experiment utilized the technique of Ripperton in which the concentration of the cation was reduced by successively washing the swollen starch by decantation after a settling period of 24 hours. The starch and washing liquid were thoroughly mixed by inverting the cylinder containing the starch and liquid several times after removal of the supernatant liquid. Care was taken to

prevent loss of starch during decantation. Two electrolyte treatments were used, with salts of different cation valency, and two wheat starches. One series of washings of each treatment for each starch was made with 0.01 N sodium citrate and one with distilled water. Three successive washings were made on each starch treatment. It was found that as the cation concentration was gradually reduced the swelling power decreased (Table 7). Sodium citrate, which contains a trivalent anion, reduced the swell more rapidly than distilled water, owing probably to the effect of the negative ion in neutralizing the positive charge contributed by the cation added at cooking. The lowered swell after three washings, as compared with the untreated starch, for the $MgSO_4$ treatments (Table 5) may be due to removal of part of the electrolyte originally present in the starch.

SUMMARY

A series of experimentally prepared wheat starches was examined in respect to the effect of wheat maturity at harvest upon starch swelling power, viscosity, and gel strength. It was found that as the wheat matured, swelling power decreased irrespective of variety or environmental conditions under which the wheat was grown. Gel strength and viscosity, however, tended to increase with ripeness, but the data collected were not as extensive as for swelling power. These changes in starch properties were associated with marked increases in test weight per bushel and per cent of vitreous kernels, and decreases in wheat moisture content.

The "amylodextrin" fraction of wheat starch was found to increase the nitrogen content and viscosity of starch solutions but to lower the pH, gel strength, and swelling power of starch.

Surprisingly little difference was noted among wheat, rice, and barley starches in respect to viscosity and swell. Potato starch, however, had much higher viscosity and swell than the cereal starches. Treating the starches at cooking time with 0.001 N electrolyte markedly decreased the viscosity and swell of potato starch, this effect increasing with the valency of the cation. When $Th(NO_3)_4$ was used, the swell was greater than when $Al_2(SO_4)_3$ was used. For cereal starches, swelling power increased with the valence of the cation, the average swell with $Th(NO_3)_4$ being more than twice that of the untreated control. It is highly probable that these differences are closely associated with changes in the electrokinetic potential of the double layer surrounding the starch particles.

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PASTEURIZATION OF LIQUID-EGG PRODUCTS.

I. BACTERIA REDUCTION IN LIQUID WHOLE EGG AND IMPROVEMENT IN KEEPING QUALITY[1]

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The production of liquid-egg products for freezing and drying has been increasing rapidly in the United States for the past 30 years. Radabaugh (1939) reported an increase from 46,000,000 pounds in 1922 to 225,000,000 pounds in 1937. The United States Department of Agriculture (1945) reported the production of 1,575,000,000 pounds of liquid egg in 1944. Of this amount, 515,000,000 pounds were used as frozen eggs, 1,025,000,000 pounds for production of dried-egg powder, and 35,000,000 pounds for immediate consumption.

One of the major problems in the production of liquid egg has been to produce a product that will not become inedible in a short time. Stiles and Bates (1912), Pennington and Stocking (1915), Redfield (1920), Brownlee and James (1939), Nielsen and Garnatz (1940), Schneiter, Bartram, and Lepper (1943), Lepper, Bartram, and Hillig (1944), and others have shown that the number and kind of bacteria present in liquid egg influence greatly its keeping quality. Their studies show that freezing and storage reduce the bacterial content of liquid egg. However, as shown by Brownlee and James (1939) and Schneiter, Bartram, and Lepper (1943), many bacteria survive freezing and storage and multiply rapidly during the defrosting of frozen egg. The growth and multiplication of bacteria in liquid egg lower the quality of the product. Objectionable odors and flavors soon appear. After a short while, depending on the holding temperature, the product may become sour, putrid, or may coagulate.

Heat treatment (pasteurization) which has been employed many years for killing objectionable bacteria in milk, cream, beer, wine, fruit juices, etc., has been considered only recently for killing bacteria in liquid egg. The general belief has been, as expressed by Epstein (1941), that liquid egg could not be heated at a sufficiently high temperature or for a sufficient length of time to kill bacteria, without causing coagulation of the proteins or otherwise impairing the culinary properties of the egg.

Van Oijen (1940) and Wedemann (1940) heated liquid duck eggs at 65°C.(149°F.) for 20 minutes for the destruction of paratyphoid and enteritidis groups of bacteria, which are sometimes found in duck eggs. Wedemann suggested the addition of a two-per cent solution of sodium citrate to retard coagulation.

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Funk (1943) reduced the bacterial content of shell eggs by immersion and rotation of the eggs in an oil bath at 60°C.(140°F.) for 10 minutes. The heat treatment also caused the eggs to retain their desirable physical properties much longer than untreated eggs.

Romanoff and Romanoff (1944) reported that dipping shell eggs in boiling water, for a period of five seconds, improved their keeping quality without impairment of the beating properties of the albumen. The treatment probably killed most of the bacteria on the surface of the egg shells and prevented penetration of microorganisms.

McFarlane, Watson, and Goresline (1945) added bacteria to liquid whole egg and heated it in a Votator heater at temperatures from 54.4 to 66.1°C.(130 to 151°F.) for approximately three seconds. The higher the temperature the greater the destruction of bacteria. A temperature of 65.6°C.(150°F.) reduced the bacterial plate count to less than 15,000 per gram or a reduction of more than 99 per cent. They stated that if liquid egg was to be heated for effective bacterial destruction, as well as for more efficient drying, it should be heated above 57.2°C.(135°F.).

Since the limited data available indicated that liquid whole egg might be heated at sufficiently high temperatures and for sufficient lengths of time to destroy most of the bacteria present, without injury to its value for food preparation, it was deemed advisable to investigate further the pasteurization of liquid-egg products.

Our objectives have been to determine the influence of such pasteurization on the following: (1) reduction of bacterial content and improvement of keeping quality; (2) physical and chemical properties; (3) value for food preparation; (4) destruction of pathogenic bacteria.

This paper is the first of a series to cover the above main objectives. In it our attempts have been to show the effects of temperature and time of heating liquid whole egg on: (a) reduction of standard plate bacterial count; (b) destruction of coliform bacteria; (c) length of time that the product remains edible.

EXPERIMENTAL PROCEDURE

Using an experimental pasteurizer (Fig. 1) the liquid egg was held in a sterile filter flask and was forced through the heater and holder by means of air pressure. The rate of passage was varied by varying the air pressure. The pressure was measured by a mercury manometer attached to the pressure line before entrance to the flask.

The liquid egg was heated as quickly as possible to the desired holding (pasteurizing) temperature by passage through eight feet of bent, thin glass tubing (three mm. outside diameter and 0.75 mm. wall thickness) submerged in a constant-temperature water bath. It was possible to heat the liquid egg from room temperature, 21.1°C.(70°F.), to 62.8°C.(145°F.) during passage through the eight feet of tubing in four seconds, when the temperature of the water bath was 65°C.(149°F.). The temperature to which the egg was heated as well as the time required to raise it to a given temperature were varied by changing the temperature of the water bath, the length of glass tubing in the heater, and the rate of passage through it. The temperature of the egg at the end of the glass-tubing heater and before entrance to the holder (pasteurizer) was measured by introducing

a copper constantan thermocouple wire through a glass T-tube and attaching it to a Leeds and Northrup potentiometer indicator.

The holder or pasteurizer consisted of a DeKhotinsky constant-temperture water bath and wire (hardware cloth) cylinders, wrapped with varying lengths of thin, pure, gum-rubber tubing (inside diameter $\frac{1}{8}$ inch and wall thickness $\frac{3}{64}$ inch. The cylinders were joined by coupling adjacent ends of the rubber tubing with a short piece of glass tubing. They were immersed in the water bath. The time the egg was held at a given holding temperature was measured by the time required for the egg to pass through different lengths of tubing. Samples were obtained at the different time intervals by lifting the glass-tube connections out of the bath, drying and loosening the connection, and collecting a small amount of egg in a sterile glass tube.

The pasteurized egg was cooled by collecting the sample in a small, thin-walled, sterile glass tube held in a beaker of ice water. The egg flowed down the side of the cold tube and was cooled to 70°F. almost instantly. Samples were kept in ice water until plated, which was always within a few hours. When larger samples were needed for keeping-quality or baking tests, the egg was cooled by passing it through glass tubing (similar to that used in the preheater) which was submerged in ice water.

The time required for the egg to pass through the heater and different lengths of tubing in the holder was measured with the apparatus filled with egg and operated at the desired temperature and pressure. To determine the time for a certain sample of egg to travel a given distance an air bubble was introduced in the line, at the connection between the flask and the heater, by loosening the rubber-tube connection momentarily to allow a little egg to flow back into the flask. The time required for the air bubble to pass through the heater and different distances in the holder was then measured.

The apparatus was cleaned by forcing a five-per cent solution of sodium chloride through it immediately following the egg. The salt solution was followed with distilled water. The tubes from the heater and coils of tubing from the holder were removed and sterilized in a steam autoclave. Before forcing the egg through the reassembled sterile apparatus, a flask of sterile water was forced through it to test for leaks, obstructions, and sterility. A sample of the wash was plated to test for sterility.

Commercial liquid-egg samples were obtained from three egg-breaking and drying plants for pasteurization in the experimental laboratory pasteurizer. The samples were taken from the holding vats [1.1 to 2.2°C.(34 to 36°F.)] after agitation. They were held in quart glass jars in a partially or completely frozen state by means of dry ice and refrigeration until defrosted, homogenized, filtered, and pasteurized. The samples were defrosted as quickly as possible by warming the jars in an incubator at 37°C.(98.6°F.) or in a pan of warm water with frequent agitation. The sample was then homogenized through a sterile hand homogenizer and allowed to flow through two layers of sterile cheesecloth into a half-gallon, sterile, glass fruit jar. The sample was thoroughly shaken in the jar; a small portion was removed for bacteriological analysis; and the remainder was transferred to a sterile filter flask for immediate pasteurization.

Unheated, heated, and heated and held liquid-egg samples were obtained from three drying plants where plate heaters were being used to heat the liquid egg to facilitate egg drying and to reduce the bacterial content of the resulting powder.

The unheated samples were taken from the line before entering the plate heater. The heated samples were taken from the line after passage through the heater but before entering the holder or pressure pump. Heated and held samples were taken from the line after passage through the holder and pressure pump. All samples were collected in small, screw-

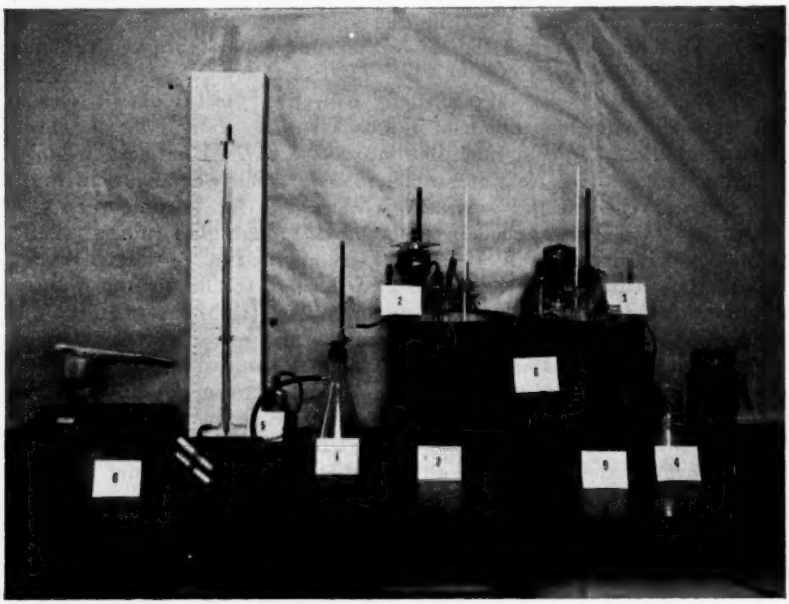

FIG. 1. Experimental pasteurizer: 1. Filter flask for holding unheated egg. 2. Preheater, consisting of glass tubing, 7, suspended in a constant temperature bath. 3. Holder, consisting of cylinders wound with rubber tubing, 9, suspended in a constant temperature bath. 4. Cooler, consisting of a sample collecting tube suspended in a beaker of ice water. 5. Mercury manometer for measuring the air pressure used in forcing the egg through the pasteurizer. 6. Potentiometer indicator for measuring the temperature of the egg at the T-tube connection, 8, between the preheater and holder.

capped, glass tubes and partially or completely frozen as quickly as possible with dry ice. The samples were kept in the frozen state until defrosted for bacteriological analysis, which was usually within 24 hours.

Standard plate counts were made according to the method of the American Public Health Association (1941). Tryptone-glucose-extract agar was used for plating and the plates were incubated at 32°C.(89.6°F.) for 72 hours. Coliform counts were made by plating on desoxycholate agar, according to the method of Leifson (1935) and the Quartermaster Corps (1944).

TABLE 1

Bacterial Content of Liquid Whole Egg

Date	Sample		Bacteria (thousands per ml.)	
	Number	History	Plate count	Coli-forms
4-15-44	1	Fresh	288	41.2
	2	Fresh; held 12 hr. at 36°F.	1,121	123.5
5- 4-44	3	Fresh	1,000	58.5
	4	Fresh; held 12 hr. at 36°F.	717	74.5
	5	Fresh; held 24 hr. at 42°F.	2,297	36.0
	6	No. 4 held 24 hr. at 42°F.	4,320	48.8
5- 9-44	7	Fresh	1,117	85.3
	8	No. 7 held 24 hr. at 42°F.	3,735	83.0
6- 3-44	9	Fresh; held 12 hr. at 36°F.	8,095	425.0
6- 8-44	10	Fresh; held 12 hr. at 42°F.	5,030	129.5
6-16-44	11	Fresh; Vat 1	947	83.6
	12	Fresh; Vat 2	763	40.3
6-20-44	13	Fresh	4,059	316.0
11-23-44	14	Defrosted	2,018	35.8
11-27-45	15	Defrosted; Vat 1	556	15.7
	16	Defrosted; Vat 2	640	17.0
	17	Defrosted; Vat 3	655	12.4
	18	Defrosted; Vat 4	415	6.8
11-28-44	19	Defrosted; Vat 1	610	10.6
	20	Defrosted; Vat 2	945	20.4
	21	Defrosted; Vat 3	1,360	29.0
	22	Defrosted; Vat 4	1,710	28.4
11-29-44	23	Defrosted; Vat 1	825	9.7
	24	Defrosted; Vat 2	3,810	56.5
1- 9-45	25	Defrosted	895	56.5
	26	No. 25 held 6 days at 42°F.	2,890	55.0
1-13-45	27	Defrosted; Vat 1	1,205	6.8
	28	Defrosted; Vat 2	516	4.1
1-19-45	29	Defrosted	402	21.9
1-21-45	30	Defrosted	142	3.7
	31	Fresh	548	0.3
	32	No. 30 held 24 hr. at 42°F.	400	1.8
2-27-45	33	Fresh	295	6.0
	34	No. 33 held 24 hr. at 42°F.	525	3.4
3-14-45	35	Fresh; held 48 hr. at 42°F.	136	9.0
4-24-45	36	Fresh	610	0.3
4-25-45	37	Fresh; held 24 hr. at 42°F.	1,035	11.5

The keeping quality of some of the unheated and pasteurized samples was studied by dividing the samples, after bacteriological analysis, into two equal parts in sterile culture tubes, plugging with cotton and storing one tube of each sample in a household refrigerator and the other one in the laboratory at room temperature. The samples were examined at intervals for signs of unwholesomeness, as indicated by a sour or putrid odor, great increase in bacterial content, change in pH or coagulation.

RESULTS

The bacterial content of 37 samples of commercial liquid whole egg (Table 1) indicates that the standard plate count varied from 136,000 to

8,095,000 per gram with an average of 1,531,000. The coliform count varied from 300 to 425,000 per gram with an average of 53,200. The low counts were from eggs broken during the late winter and early spring while the high counts were from those broken during the late spring and early summer. In general, high coliform counts were associated with high standard plate counts and vice versa. Holding liquid egg overnight in a

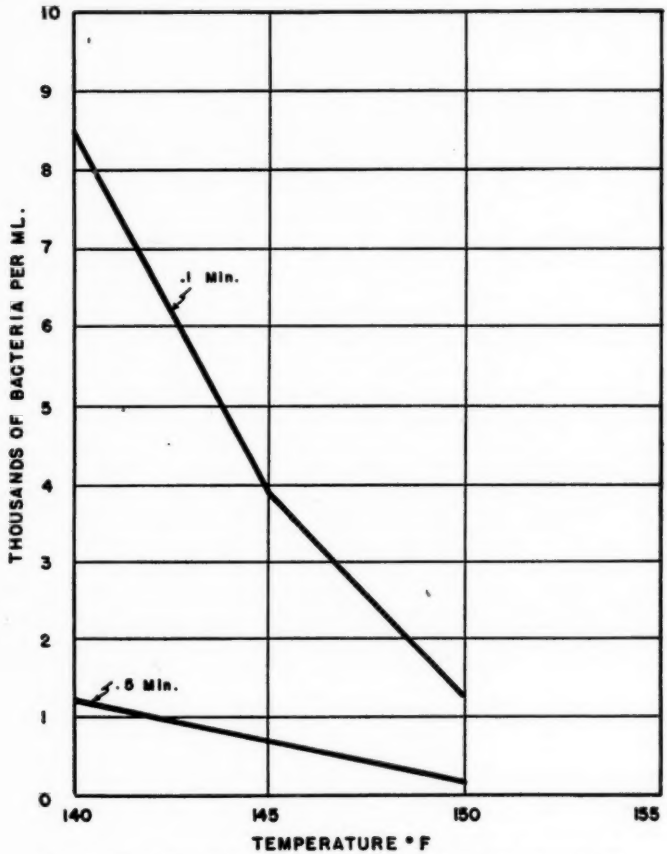

FIG. 2. Short-time pasteurization of liquid whole egg, Sample 35.

holding vat at 1.1 to 2.2°C.(34 to 36°F.) or in a household refrigerator at 4.4 to 7.2°C.(40 to 45°F.) resulted in marked increase in standard plate count and some decrease in coliform count. Defrosted eggs contained less bacteria than eggs broken in the late spring but more than eggs broken in the late winter.

Twenty-seven samples of commercial liquid whole egg were pasteurized in the experimental laboratory pasteurizer. The tests may be divided into two groups on the basis of heating time. Samples heated less than one

TABLE 2

Short-Time Pasteurization of Liquid Whole Egg

Pasteurization		Standard plate count (thousands per ml.)					Coliforms (thousand per ml.)				
Temp.	Time	Trials	Av.	High	Low	Reduction	Total trials	Coli present in	Av.	High	Low
°F.	*min.*					*pct.*					
Unheated	.....	15	1,533.9	12,500.0	136.0	.....	13	13	51.5	425.0	1.4
130	.1-.3	8	290.1	845.0	4.1	91.5	6	5	1.7	6.2	0.0
135	.1-.3	7	22.1	69.5	0.6	96.2	6	3	1.0	4.0	0.0
	.4-.5	2	3.0	3.6	2.5	98.4	2	1	0.2	....	....
140	.1-.3	10	4.5	13.2	0.2	99.1	8	4	0.3	1.1	0.0
	.4-.6	4	11.7	24.1	1.2	98.3	4	0	....	....	....
142	.1-.3	4	4.2	7.6	0.9	99.6	2	1	0.3	....	....
	.4-.6	4	9.6	18.2	1.0	98.5	2	0	....	....	....
144	.1-.3	5	2.8	6.3	0.2	99.7	4	2	0.9	1.9	0.01
	.4-.6	3	13.0	21.4	1.0	98.1	3	1	0.01	....	....
145	.1-.3	6	1.9	6.6	0.1	99.4	3	1	0.4	....	....
146	.1-.3	3	4.2	5.7	1.3	99.0	2	2	0.3	0.6	0.1
	.4-.5	4	0.6	0.7	0.4	99.7	2	0	....	....	....
147	.2	2	15.6	22.7	8.6	99.7	2	2	0.2	0.3	0.1
	.8	1	11.7	....	....	98.9	3	0	....	....	....
148	.1-.3	3	1.4	2.8	0.2	99.9	1	1	0.9	....	....
	.4-.5	2	0.4	0.5	0.3	99.8	1	0	....	....	....
150	.1-.3	6	1.2	4.3	0.0	99.9	3	1	0.1	....	....
	.5	1	0.2	....	....	99.9	1	0	....	....	....
152	.1-.2	2	0.3	0.5	0.2	99.9	1	1	0.1	....	....
154	.1	1	0.4	....	....	99.9	1	0	....	....	....
155	.1-.2	4	0.4	1.6	0.0	99.9	2	0	....	....	....
156	.1	1	0.2	....	....	99.9	1	0	....	....	....
160	.2	1	0.2	....	....	99.9	1	0	....	....	....
	.5	1	0.2	....	....	99.9	1	0	....	....	....

minute are here regarded as short-time pasteurized. Those heated longer than one minute are regarded as holder pasteurized.

The short-time pasteurization data are summarized (Table 2); the pasteurization time includes the time required to heat the liquid egg to

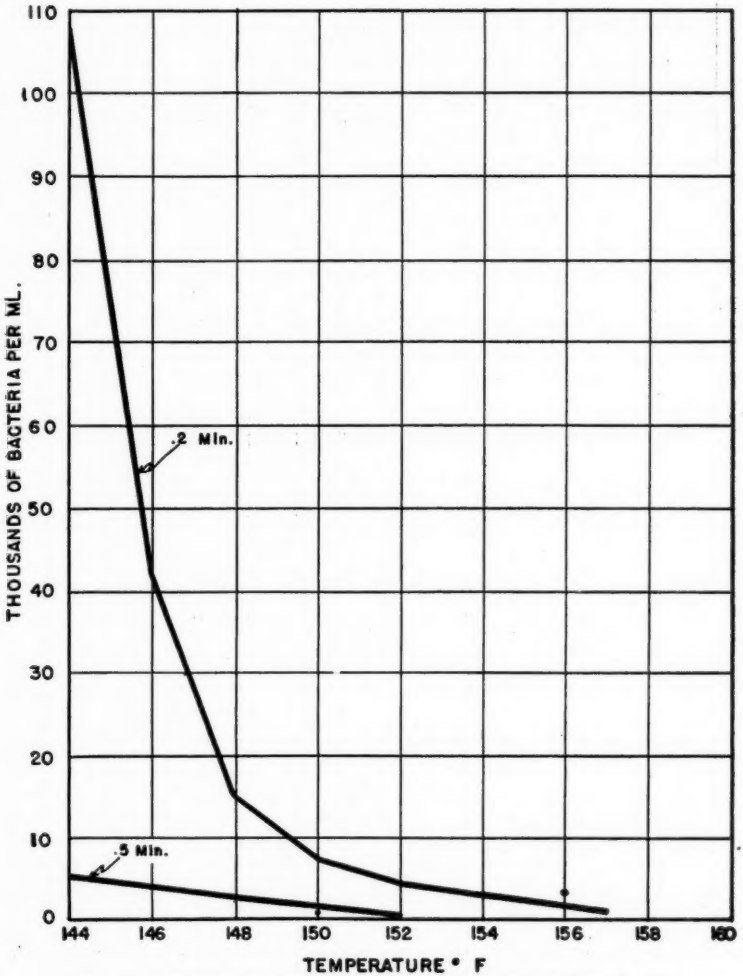

FIG. 3. Short-time pasteurization of liquid whole egg, Sample 37.

the temperature of the holder or pasteurizer. This preheating time varied from 0.1 to 0.2 minute. Thus, in some of the tests there was practically no holding time.

The 15 samples of unheated egg used for the short-time pasteurization tests varied widely in bacterial content. The number of bacteria surviving

pasteurization also varied widely in the different samples. The same sample of egg was not used for all of the temperatures and time ranges shown (Table 2). This would account for the variations in survival of bacteria shown for a given temperature and time. It would also account for the apparent greater survival of bacteria when heated at 63.9°C.(147°F.) for 0.2 minute than at 62.8°C.(145°F.) for 0.1 to 0.3 minute.

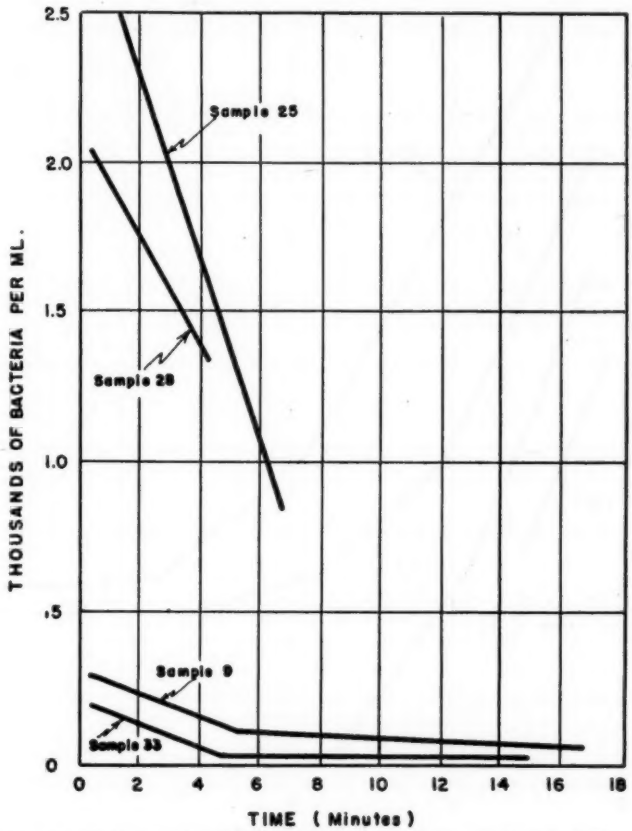

FIG. 4. Holder pasteurization of liquid-whole-egg samples at 140°F.

In order to save space all of the pasteurization tests made with only two of the samples have been presented separately (Figs. 2 and 3). They show a wide variation in the heat resistance of the bacterial flora in the two samples. These charts also show that both temperature and time are important factors for bacterial destruction in liquid whole egg.

In nearly all cases pasteurization of liquid whole egg at 145°F. or higher, for 0.1 to 0.3 minute or longer, reduced the standard plate count to less than 5,000 per ml. or more than 99 per cent. In about 50 per cent of the samples a few coliforms survived pasteurization at 145 to 147°F.

for 0.1 to 0.3 minute. None of the coliforms survived a temperature of 67.8°C.(154°F.) for 0.1 to 0.2 minute.

The holder pasteurization data are summarized (Table 3) on 12 samples of egg used for the tests. They varied widely in bacterial content. A tem-

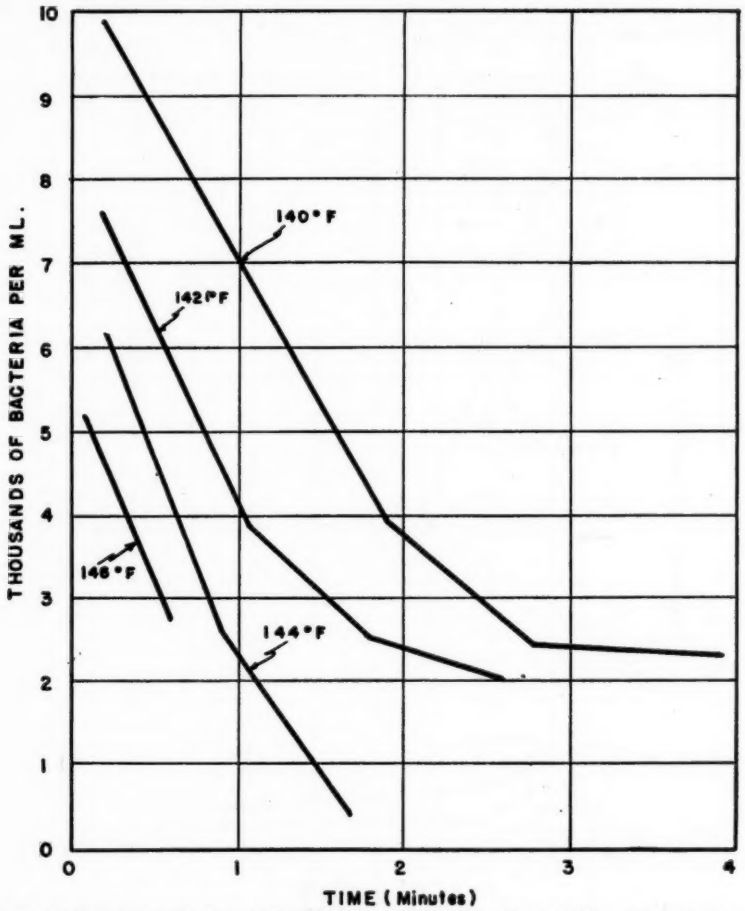

FIG. 5. Holder pasteurization of liquid whole egg, Sample 37, at different temperatures.

perature of 60°C.(140°F.) for 10 minutes, 61.1 to 62.2°C.(142 to 144°F.) for 6 minutes, or 62.8°C.(145°F.) for one minute reduced the standard plate count below 4,000 per ml. or more than 99 per cent. These temperatures and times also destroyed all of the coliforms in the 12 liquid-whole-egg samples.

The bacterial flora of the holder-pasteurized samples varied widely in heat resistance (Fig. 4 and Table 3). Data from one sample pasteurized by the holder method is presented separately (Fig. 5). The higher the

TABLE 3

Holder Pasteurization of Liquid Whole Egg

Pasteurization		Standard plate count (thousands per ml.)					Coliforms (thousand per ml.)				
Temp.	Time	Trials	Av.	High	Low	Reduction	Total trials	Present in	Av.	High	Low
°F.	min.					pct.					
Unheated		12	5,136.0	20,200,0	141.0	.....	12	12	13.4	56.5	.3
135	1- 9.9	4	0.03	0.7	0.0	99.9	4	0	....	....	....
	10-19.9	2	0.15	0.2	0.1	99.9	....	....	....	....	....
138	1- 4.9	5	24.3	34.5	17.9	97.9	5	0	....	....	....
	5- 9.9	4	15.3	16.7	13.6	98.3	4	0	....	....	....
140	1- 4.9	16	8.7	20.8	0.03	99.1	14	0	....	....	....
	5- 9.9	4	6.3	15.8	0.4	99.6	4	0	....	....	....
	10-19.9	6	0.5	2.5	0.0	99.9	4	0	....	....	....
	20-32.7	3	0.3	0.4	0.2	99.9	3	0	....	....	....
142	1- 2.9	7	9.4	20.8	0.1	99.0	5	0	....	....	....
	3- 5.9	3	9.1	15.1	0.5	99.4	3	0	....	....	....
	6-10.4	2	1.5	3.0	0.0	99.9	2	0	....	....	....
144	1- 2.9	4	7.5	11.8	0.4	98.4	4	0	....	....	....
	3- 5.9	5	8.8	19.4	0.4	99.4	5	0	....	....	....
	6- 9.9	3	1.2	3.5	0.01	99.8	3	0	....	....	....
	10-28.4	4	0.01	0.01	0.0	99.9	1	0	....	....	....
145	1- 5.9	2	0.08	0.1	0.06	99.9	1	0	....	....	....
	6-30.2	3	0.0	....	....	100.0	3	0	....	....	....
146	2.7	1	0.3	....	....	99.9	1	0	....	....	....
147	3.7	1	3.0	....	....	99.7	1	0	....	....	....

temperature used, the shorter the time required to reduce the bacterial content below 5,000 per milliliter.

Thirty-three tests were made on the bacterial count of liquid whole egg before and after heating in plate heaters in three different plants. The time required for the egg to pass through the heaters was not known definitely but it was estimated to be about 0.7 minute. It probably reached the pasteurization temperature when about halfway through the heater. Plant practices made it difficult to obtain definite temperature and time effects on the bacterial content of the egg being heated, before drying. For instance, some of the heated egg was by-passed or recirculated back with the cold egg and reheated. The rate of flow of egg through the heater as well as the temperature of the surrounding hot-water jacket were varied, depending on the operation of the dryer and the film formed on the inside of the heater.

Sixteen tests were made in Plant A. They covered a period of about a year. At first the egg was heated to about 57.8°C.(136°F.), and later it was heated to 61.7°C.(143°F.). Still later a holding tank was added to hold the egg close to 143°F. for about six minutes. Finally, the small heater and tank were replaced by a much larger heater in which two to two and one-half minutes were required for the egg to pass through it. The effects of these changes on the average bacterial content of the egg after heating may be summarized as follows:

Sample	Plate count			Coliforms	
	Number of trials	Thousands per gm.	Reduction per cent	Thousands per gm.	Reduction per cent
Heated to 136°F.	5	43.6	95.2	1.9	99.0
Heated to 143°F.	6	118.3	96.6	0.1	99.4
Heated to 143°F. and held 6 minutes in a tank	6	106.7	98.3	0	100.0
Heated to 143°F. for about 2 min. in big heater	3	2.0	99.7	0	100.0

Thirteen tests were made in Plant B. At first this plant was heating the egg to 55°C.(131°F.). Later the temperature was increased to 143°F. No holder was in use at the time these tests were made. However, in two tests, samples were taken about 50 feet beyond the pressure pump, at the entrance to the dryer, as well as at the end of the heater. The additional time required for the egg to reach the dryer would correspond to a short holding period of probably 0.1 minute. The effects of the increased temperature and the short holding period on the average bacterial content of the heated egg may be summarized as follows:

Sample	Plate count			Coliforms	
	Number of trials	Thousands per gm.	Reduction per cent	Thousands per gm.	Reduction per cent
Heated to 131°F.	3	77.2	97.8	4.8	94.9
Heated to 143°F.	7	39.2	97.0	0.09	99.6
Heated to 143°F. and held 0.1 minute	2	41.3	98.3	0.005	99.9

Four tests were made in Plant C. After the egg was heated to 142°F. in a plate heater it was held at this temperature for about two minutes by passage through a 48-tube milk heater. The additional heating or holding period reduced the standard plate count from an average of 12,000 to 7,000 per gram and practically eliminated the few remaining coliforms.

Eleven different lots of unpasteurized and pasteurized liquid whole egg were observed for keeping quality. Some of the typical data obtained are summarized (Tables 4 and 5).

The unheated, liquid-whole-egg samples became sour within 24 hours when held at room temperature (70°F.) and within three to seven days when held in a household refrigerator (42 to 45°F.). The average of the pH reading changed from about 7 before any holding period to 6.7 when held three to seven days at 42°F. and to 6.1 when held one day at 70°F. The bacterial content of the unheated egg increased greatly during three to seven days' storage at 42°F. No attempt was made to measure the increase during one day of storage at 70°F., since there is already ample data available to show that the increase is very great.

Short-time pasteurization (Table 4) had no appreciable influence on the time that liquid whole egg remained normal, as measured by odor, appearance, and pH when the samples were held at 70°F. Samples heated for 0.1 to 0.3 minute at temperatures up to and including 150°F. became sour, while those heated above 150°F. coagulated.

Short-time pasteurized samples, at 145°F. or above for 0.1 to 0.3 minute, generally remained normal for at least a week when held in a household refrigerator at 42°F. When samples were heated at 155°F. for 0.1 to 0.3 minute, they remained normal for at least two weeks when held at 42°F. They were not observed over a longer period.

Holder pasteurization (Table 5) did not prolong the keeping time of liquid-whole-egg samples to any appreciable degree when they were held at room temperature (70°F.). Nearly all of the samples coagulated within 24 hours. A few of them became sour. The pH of the coagulated samples ranged from 5.5 to 6.1.

Samples pasteurized at 140°F. for 10 minutes, 142°F. for six minutes, or 144° F. for two minutes usually remained normal, as judged by bacterial content, odor, appearance, and pH, when held for at least two weeks in a household refrigerator (42°F.).

DISCUSSION

The microbiology of liquid egg is much like that of milk. This is indicated by the somewhat similar specifications for milk, Milk Ordinance (1939), and eggs, Quartermaster Corps (1944). The bacterial content of both products depends largely on the sanitation practices used in handling and the holding temperature.

We were able to pasteurize liquid whole egg at the temperature and for as long as, or longer than, the time recommended for milk by the holder method (Table 3).

When the recommended short-time pasteurization of milk was attempted with egg, some coagulation trouble was encountered. This trouble could be overcome by using glass tubing of greater internal diameter and more

TABLE 4

Influence of Short-Time Pasteurization on Keeping Quality of Liquid Whole Egg

Number	Samples				Observations		
	Treatment		Storage				
	Temp.	Time	Temp.	Time	Bacteria (thousands per ml.)	Condition	pH
	°F.	*min.*	°F.	*days*			
33	Unheated	....	....	0	295.0	.....	....
			42	3	14,700.0	Sour	7.3
			70	1	.....	Sour	....
	145	.2	....	0	0.4	.....	....
			42	8	645.0	Sour	6.9
	150	.2	....	0	0.04	.....	....
			42	8	1.3	Normal	7.1
			42	16	.....	Sour	....
	155	.2	....	0	0.04	.....	....
			42	8	0.1	Normal	7.5
			42	16	.....	Normal	....
34	Unheated	....	....	0	525.0	.....	....
			42	17	144,500.0	Sour	6.7
	142	.3	....	0	1.5	.....	....
			42	13	0.3	Normal	7.4
	148	.3	....	0	1.3	.....	....
			42	13	0.2	Normal	7.5
35	Unheated	....	....	0	136.0	.....	8.0
			42	6	121,500.0	Sour	6.8
			70	1	.....	Sour	....
	142	.5	....	0	1.0	.....	7.2
			42	6	11.5	Normal	7.3
			70	3	.....	Sour	....
	146	.5	....	0	0.6	.....	7.2
			42	6	4.4	Normal	7.2
			70	3	.....	Sour	....
	150	.5	....	0	0.2	.....	7.0
			42	6	0.1	Normal	7.2
			70	3	.....	Sour	....
37	Unheated	....	....	0	1,035.0	.....	....
			42	13	421,000.0	Coagulated	6.1
			70	1	.....	Sour	....
	144	.2	....	0	108.5	.....	....
			42	13	1,900,000.0	Sour	6.3
			70	1	.....	Sour	....
	148	.2	....	0	14.9	.....	....
			42	13	183,500.0	Sour	6.9
			70	1	.....	Sour	....
	152	.2	....	0	4.6	.....	....
			42	13	902,500.0	Sour	7.0
			70	1	.....	Sour	....
	157	.2	....	0	0.1	.....	....
			42	13	7,850.0	Normal	7.1
			70	1	.....	Coagulated	....

TABLE 5

Influence of Holder Pasteurization on Keeping Quality of Liquid Whole Egg

Number	Samples				Observations		
	Treatment		Storage				
	Temp.	Time	Temp.	Time	Bacteria (thousands per ml.)	Condition	pH
	°F.	min.	°F.	days			
	Unheated	.....	....	0	9,500.0	.....	....
			42	7	111,000.0	Sour	6.8
38	140	32.7	....	0	0.2	.....	....
			42	13	5.1	Normal	7.2
			70	1	.....	Coagulated	....
	140	10.5	....	0	2.5	.....	....
			42	13	0.1	Normal	7.2
	Unheated	.....	....	0	11.7	.....	7.3
			42	6	305,200.0	Sour	6.4
			70	1	.....	Sour	....
	140	2.8	....	0	0.05	.....	7.3
39			42	6	1.1	Normal	7.5
			70	1	.....	Coagulated	....
	142	1.6	....	0	0.1	.....	7.2
			42	6	1.9	Normal	7.3
			70	1	.....	Coagulated	....
	Unheated	.....	....	0	12,500.0	.....	6.7
			42	14	1,650,000.0	Sour	6.4
			70	1	.....	Sour	6.2
	140	16.9	....	0	0.0	.....	6.7
			42	13	2.0	Normal	6.7
			70	1	.....	Coagulated	6.0
	140	7.4	....	0	0.4	.....	6.8
			42	13	284,000.0	Sour	6.7
40			70	1	.....	Coagulated	5.6
	142	6.0	....	0	0.3	.....	6.7
			42	13	1.8	Normal	6.7
			70	1	.....	Coagulated	5.5
	142	3.0	....	0	0.9	.....	6.7
			42	13	1.1	Sour	6.7
			70	1	.....	Coagulated	5.7
	144	4.8	....	0	0.4	.....	6.7
			42	13	8.5	Sour	6.7
			70	1	.....	Coagulated	5.7
	Unheated	.....	....	0	1,035.0	.....	....
			42	13	421,000.0	Coagulated	6.1
			70	1	.....	Sour	....
	140	3.9	....	0	2.3	.....	....
41			42	13	10,500.0	Normal	7.2
			70	1.5	.....	Coagulated	....
	144	1.7	....	0	0.4	.....	....
			42	13	2,164.5	Normal	7.2
			70	1.5	.....	Coagulated	....
	Unheated	.....	....	0	610.0	.....	7.0
			42	13	2,450,000.0	Sour	6.6
			70	1	.....	Sour	6.1
42	144	5.7	....	0	0.02	.....	7.0
			42	13	7.2	Normal	7.2
			70	1.5	.....	Coagulated	6.1

of it in the preheater. However, egg pasteurized at 148 to 150°F. for 0.5 minute or 152 to 155°F. for 0.1 to 0.2 minute contained no appreciably greater number of bacteria than egg pasteurized at 160°F. for 15 seconds (Table 2).

Plant heating of liquid egg to 143°F. for about 0.7 minute reduced the plate count to an average of about 118,000 per gram or a reduction of about 96 per cent. When an approximate two-minute holding period was used, the plate count was reduced to less than 2,000 per ml., or a reduction of more than 99 per cent. It appears that practical plant heating of liquid egg can be carried out as effectively for the destruction of bacteria as tests conducted under laboratory conditions.

All of the unheated, liquid-whole-egg samples contained coliforms (Table 1). In most of the samples they were easily killed by pasteurization. No coliforms survived pasteurization by the holder method (Table 3). They were not killed as easily by the short-time pasteurization (Table 2). A few coliforms survived temperatures at 150 and 152°F. for 0.1 to 0.3 minute. Two strains of *Escherichia coli*, differing widely in heat resistance, appeared to be killed more easily in liquid whole egg than in milk. Details regarding these and other coliform studies in egg products will be presented in a later paper.

Souring of samples pasteurized at temperatures below 152°F. for 0.1 to 0.3 minute and coagulation at 152°F. for 0.2 to 0.5 minute (Table 4) and by the holder method (Table 5), may have been due to the type of bacterial flora surviving the different pasteurization temperatures and time. Gibbons and Fulton (1943) have shown that the pH of some liquid-whole-egg samples decreased at first and then increased during storage. Other samples continued to decrease in pH until coagulation took place (pH about 5.7). It is possible that pasteurization may hasten the spoilage of some samples that are held at room temperature. This would depend on the bacterial flora present in the unheated, liquid whole egg. Destruction of the easily killed proteolytic bacteria might permit the more heat-resistant, acid-forming bacteria to grow unhindered and thus hasten an acid coagulation. This problem needs further study.

The data indicate that a sour, putrid or otherwise abnormal odor is generally noticeable before a marked change in pH. This observation is in agreement with the report by Lepper, Bartram, and Hillig (1944). There was a tendency for samples with a sour odor to have a pH between 6 and 7. Coagulated samples generally had a pH of 5.6 to 5.9.

SUMMARY

The bacteria in 37 liquid-whole-egg samples varied greatly in number and heat resistance.

Holder pasteurization of 11 of the 12 whole-egg samples at 140°F. for 10 minutes, 142°F. for six minutes, or 145°F. for one minute reduced the standard plate bacterial count to less than 3,000 per ml. or more than 99 per cent and eliminated all coliforms.

Short-time pasteurization of 14 of the 15 samples at 146°F. for 0.5 minute or 155°F. for 0.1 minute produced similar bacterial reductions.

The keeping time of nine samples was prolonged at least a week by pasteurization when they were held in a refrigerator.

Pasteurization had little effect on the keeping time of samples held at room temperature.

ACKNOWLEDGMENT

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PASTEURIZATION OF LIQUID-EGG PRODUCTS. II. EFFECT OF HEAT TREATMENTS ON APPEARANCE AND VISCOSITY[1]

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This is the second in a series of papers on heat treatment of liquid-egg products. The first paper, by Winter, Greco, and Stewart (1946), dealt with some of the microbiological problems involved in the pasteurization of liquid-egg products. The present paper is a preliminary study of the effect of heat treatments, in the pasteurizing range, on the appearance and viscosity of liquid-egg products: yolks, whites, and mixed yolks and whites (whole eggs).

Preheating liquids prior to spray-drying offers distinct advantages in addition to the benefits derived from killing bacteria. The decreased viscosity and surface tension brought about by heating materially help to bring about better atomization at the spray nozzle and more rapid attainment of a high egg-particle temperature during drying. These effects are important in increasing the efficiency of spray-drying and in obtaining dried whole egg of low moisture content (below three per cent), especially in the Gray-Jensen-Douthitt type of dryer.

It is obvious that coagulation is the limiting factor controlling the heat treatment of egg products. Although a great deal of work has been done on the denaturation and coagulation of purified egg proteins, particularly egg albumen, very little work has been done with the white, yolk, and liquid whole egg.

MacFarlane, Watson, and Goresline (1945) reported that the preheating temperature and time for whole egg, before drying, need to be carefully controlled over a narrow range in order to avoid bacterial growth or coagulation.

A large number of studies made on albumen show clearly that the rate of coagulation of any heat-denaturable protein is governed to a considerable degree by the following factors: temperature, time, pH, electrolytes, protein concentration, and sugar and similar materials.

For purposes of orientation in the present problem of determining the effects of heat treatment on liquid-egg products, it seemed desirable to make a study of the following problems:

A. To devise a process of heating whereby

1. Liquid egg can be heated to the desired temperatures in the shortest possible time.

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2. Liquid egg can be held at the desired temperatures for varying periods of time.
3. Heat-treated liquid egg can be cooled in the shortest possible time.

B. In addition to observing general qualitative changes, to employ a sensitive, convenient, and reproducible method for measuring the rate of denaturation of liquid-egg products.

C. To determine the effect of time and temperature on liquid-egg products, using the criteria employed.

PREPARATION OF SAMPLES

Day-old pullet eggs secured from the College poultry farm were stored at a temperature of 30°C.(86°F.) and at a relative humidity of 20 to 40 per cent for seven days. They were then refrigerated at 10°C.(50°F.) until ready for use. This storage simulated conditions to which shell eggs used in commercial freezing and drying are subjected before arriving at the breaking plant. A preliminary study indicated that there was a slower rate of denaturation in the liquid from aged eggs as compared with that from fresh eggs. Thus, there was an additional reason for controlling the age of the eggs used for this study.

Whole Egg: The eggs were broken and mixed in a Waring blender, using two-second intervals, to give a total blending time of five minutes. The short interval reduced foaming to a minimum. This procedure was found essential for breaking up the structure of the egg, thereby insuring a homogeneous sample for viscosity measurement. Commercially prepared liquid eggs are broken down thoroughly for spray-drying but not usually for freezing. The melange was then placed in the refrigerator for a few hours, and the small amount of foam was skimmed from the surface.

Egg White: The whites were separated from the yolks; they were then prepared in the same manner as the whole eggs.

Egg Yolk: The yolks were separated from the whites, and each yolk was carefully washed with running water to remove all traces of white. The yolk was then transferred from one hand to the other, and after each transfer the hand was wiped dry on a towel. This procedure removed the water resulting from the washing process. The yolk was then put into a Petri dish and allowed to stand for a few minutes, after which time the chalazae were removed with a pair of forceps, the yolk membrane broken, and the liquid yolk (free of the membrane) poured into a small fruit jar. The percentage of total solids was determined for each batch and was found to vary from 50.5 to 51 per cent. These figures agree well with those of Mitchell (1932) for similar types of eggs. The yolks were homogenized by the same procedure as that described for whites and whole eggs.

TREATMENT OF SAMPLES

Instantaneous Heat Treatment: The prepared egg sample, at 25°C. (77°F.), was brought up to the desired temperature almost instantaneously by the use of a special heat exchanger (Fig. 1). The water for heating the sample was pumped from a constant-temperature water bath through the heat exchanger. The liquid egg, in a liter suction flask, was driven through

the spiral tubing of the exchanger by the use of an air pressure of 60 centimeters of mercury. A copper-constantan thermocouple, which was connected to a potentiometer and inserted into the emerging liquid egg stream by the use of a T-tube at the outlet of the exchanger, recorded the temperature of the sample.

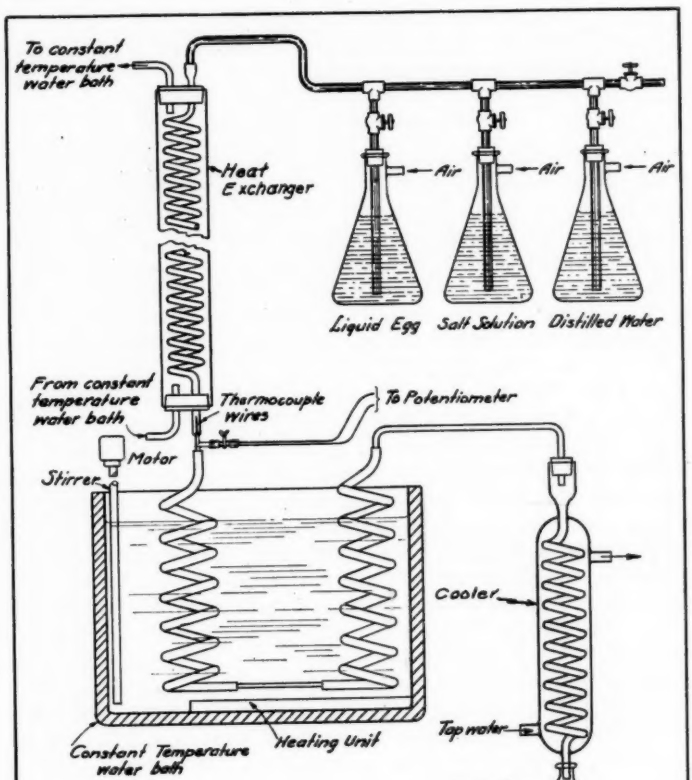

FIG. 1. Experimental pasteurizer.

For both whole-egg and white samples a differential of only 1.5°C. (between the temperature of the water bath and the final temperature of the samples) was required to bring the samples from 25°C. to the desired temperature, but for yolks it was 2°C. The time required for the heat treatment varied for the three products as follows: whole egg, 8.5 ± 0.5 seconds; egg white, 6.2 ± 0.5 seconds; egg yolk, 21 ± 1.5 seconds.

A study was made of various air pressures in order to determine the optimum air pressure for bringing the temperature of the sample from 25°C. to the desired temperature in the shortest practical time. The results

indicated that 60 centimeters mercury was the most practical one to use. It was not definitely determined whether the sample reached the final temperature before it reached the outlet of the spiral tubing, where the thermocouple was located. Therefore, the total heating time used may indicate one of two things: (1) the time required for the sample to just reach the final temperature or (2) the time required for the sample to reach the final temperature, plus some holding time at that temperature. In the latter case the time held would be so short that it would have little effect on the results.

Holding Periods: After bringing the sample up to the desired temperature, it was held for varying periods of time by continuous flow through varying lengths of rubber tubing immersed in a constant-temperature water bath (Fig. 1). The holding time was recorded by a stop watch. In order to keep the rubber tubing immersed in the bath, 12 foot-lengths were coiled around frames of mesh screen and the required lengths (for a given holding period) were joined by glass tubing.

Cooling: The heated and held sample was then cooled to approximately 20°C.(68°F.) by running it directly through a water condenser. The cooled samples were collected in prescription bottles, stoppered tightly, and stored in a refrigerator overnight.

VISCOSITY MEASUREMENTS

Viscosity was chosen as a quantitative index of denaturation in preference to the determination of solubility or sulfhydryl groups for several reasons. It can be readily employed for following the progress of denaturation of a mixture of proteins because it is a physical measurement of the effect of heat on the system as a whole; it is a sensitive criterion, making possible the detection of denaturation in the early stages; it is easily reproducible; and it is relatively simple and convenient to measure.

Some study was made on the detection of sulfhydryl groups in liquid-egg products, but the results have been very erratic. Further studies are now in progress.

Capillary Viscometer: A viscometer patterned after the one devised by Bateman and Sharp (1928) was employed (Fig. 2). It consisted of a convenient length of uniform capillary tubing, a glass tube which was stoppered at both ends after the sample had been poured into it, and a 50-milliliter, graduated centrifuge tube which served as a receiver. Rubber stoppers, metal sheets, bolts, and wing nuts were used to hold the assembly in place. The apparatus was placed in a 25° C., constant-temperature water bath equipped with a stirrer, and was connected to a compressed-air supply tank. A mercury manometer registered the pressure applied. The pressure was varied by means of a reducing valve attached to the main air-pressure line serving the laboratory.

Selection and Calibration of Capillary Tube: The detailed directions for the calibration of the viscosity apparatus, as given by Bingham (1922) and Barr (1941), were followed. The methods used for determining the mean radius of the capillary tubes were the microscopic method and the mercury-thread method. Having determined the radius, the necessary length of the capillary tube was calculated. Barr (1941) and Bingham

(1922) agree that a certain ratio should exist between the bore and the length of the capillary tube in order to minimize the error due to kinetic energy. Barr suggests the use of a capillary tube whose length is at least 300 times its radius, while Bingham suggests a length of at least 500 radii. In this study capillaries of approximately 500 radii length were used.

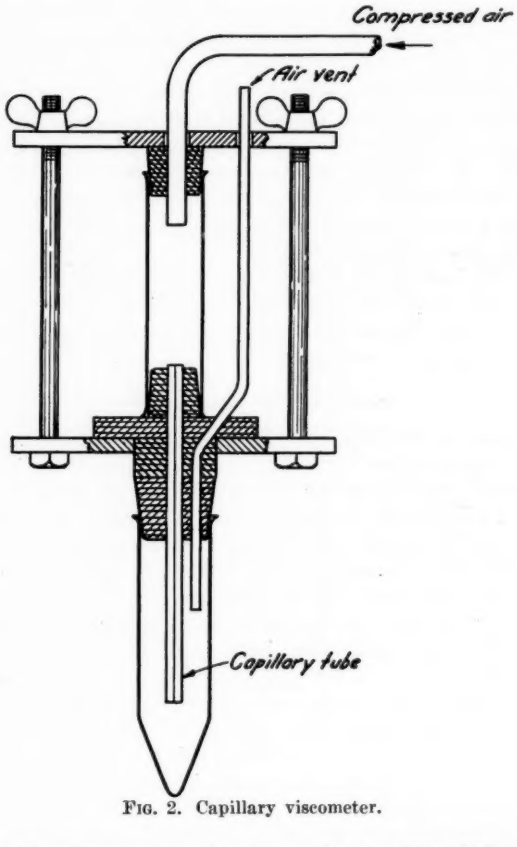

FIG. 2. Capillary viscometer.

Viscosity Data: The prepared egg sample (in 250-milliliter centrifuge bottles) was centrifuged in an International Size 2 centrifuge at a speed of 1,600 revolutions per minute for 30 minutes. The supernatant liquid was decanted and its viscosity determined. Thirty milliliters of the liquid at 25°C. were poured into the viscometer; the apparatus was then set in position and connected to the air line. A convenient number of drops was counted and the required time recorded. Preliminary work showed that recording the time for a certain number of drops was more accurate and gave more reproducible results than recording the time for the flow of a definite volume of liquid. The volume of the sample was determined from density data and the weight of the drops used.

In order to insure the absence of any peculiarities of the apparatus, the viscometer was calibrated by using 40- and 60-per cent sucrose solutions. The calculated viscosities of the sucrose solutions were found to check very well with the figures given by Bingham (1922).

The pressure (in grams per square centimeter) used for calculating the viscosity was corrected according to Bingham (1922). The corrections made were for temperature and for the hydrostatic head exerted by the liquid-egg column above the capillary tube.

All data for the study of the effect of time and temperature reported herein are the averages of four preheating trials. Viscosity data for any one trial are the averages of quadruplet determinations.

GENERAL OBSERVATIONS ON RESULTS

Whole Egg: Very few visible changes were observed during the heating of liquid whole egg. A small quantity of somewhat "powdery" sediment was obtained after centrifuging and prior to measuring viscosity. This sediment was observed in the native as well as in the heated whole egg. It may have been disintegrated yolk membranes. In addition, a small amount of gelatinous precipitate was obtained in the heated samples.

Egg White: Interesting observations were made on the product from the instantaneous heat treatment of liquid egg white at the several different temperatures. At least four phenomena were observed: turbidity, gel formation, precipitation, and coagulation. Turbidity was observed in the sample heated to 62.5°C.(144.5°F.). At 63°C.(145.4°F.), although the egg white was clear, a gelatinous mass collected on the surface of the liquid. Increased turbidity and precipitation were observed between 63 and 66°C., but at 66°C.(150.8°F.) the liquid became clear and the gelatinous mass again appeared on the surface. Above 66°C. turbidity became evident again and increased progressively until a white coagulum formed. The egg white at 70°C.(158°F.) gave off a "cooked-egg" odor and had the consistency of a soft-boiled egg.

A similar sequence of events was observed in studying the effects of holding time on the liquid egg white. After holding for 20 seconds at 62.5°C., a trace of gelatinous precipitate was obtained after centrifuging; after holding for 45 seconds, some turbidity was evident and loose gelatinous flocs occupied a large portion of the centrifuged samples. The amount of gel increased when the holding time was raised to 70 seconds. Even after centrifuging, it was very difficult to pipette out a clear supernatant liquid since the gelatinous flocs would not separate. At 90 seconds there were fewer flocs and the turbidity of the supernatant liquid had decreased. A transition occurred at 145 seconds; the gel became slightly opaque and began to differentiate into segments which, on further holding, formed into particles similar to swollen rice grains. With still further holding the gel became increasingly opaque, reaching a maximum whiteness at 445 seconds' holding time.

Egg Yolk: No visible changes occurred in the heat treatment of liquid egg yolk. The samples yielded no sediment or gelatinous material in the

centrifuge bottle. Visible changes here were undoubtedly prevented by the color and opaqueness of the material.

VISCOSITY CHANGES

The apparent viscosity obtained by the use of the capillary viscometer is expressed in centipoises. Although objection will be found to expressing the viscosity of a non-Newtonian system in terms of the absolute unit of viscosity, Robinson (1939) gives some justification for this. He states: "In the commonly accepted sense, such a quantity is not a viscosity coefficient at all, and to emphasize this, it is usually called an 'apparent viscosity'. It will still, however, be a measure of the rate of dissipation of energy in the system per unit volume and (velocity gradient)^2. Since this is what a viscometer actually measures, there may be less objection to calling it a viscosity than is often supposed."

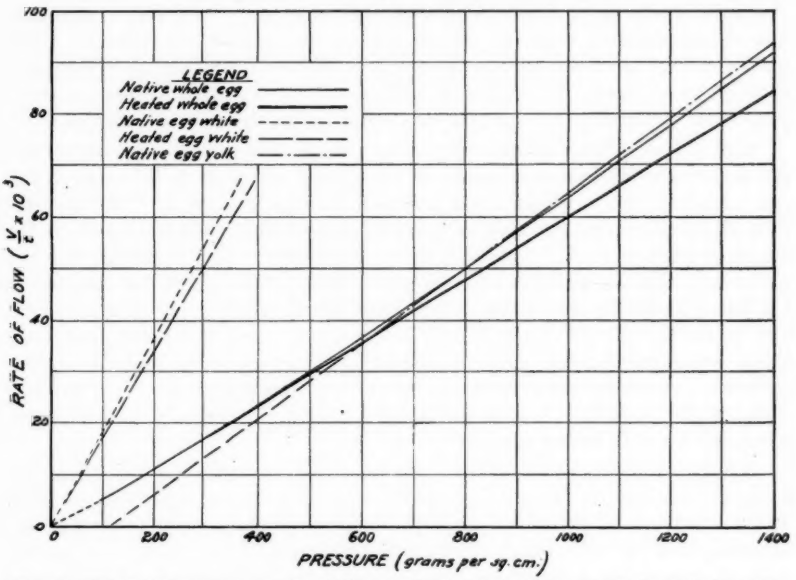

FIG. 3. Effect of pressure on rate of flow of liquid whole egg, egg white, and egg yolk.

A study was made of the effect of pressure on the rate of flow of liquid whole egg, egg white, and egg yolk in the capillary viscometer. The results (Fig. 3) show that within the limits of the data collected the rates of flow are linear with pressure.

It will be seen that by extrapolation to zero pressures the flow rates of all but the liquid yolk pass through the origin. Since the intercept made on the pressure axis in the case of yolk was obtained by extrapolation alone, it may not represent a true "yield value." It is generally agreed that at extremely low pressures the linear relationship between pressure and flow rate does not hold; the line curves toward the origin. "Yield value" may, therefore, have no real meaning. It was not considered im-

portant to establish the existence of a "yield value" for egg yolk since the main interest was not in the viscosity as such, but was rather in its rate of change with heating.

The systematic study of the effects of time and temperature on changes in viscosity to follow was made at a constant pressure for each product. The exact pressure used was selected with special regard for the accuracy of measuring the volume, per unit time, of the product passing through the capillary.

EFFECTS OF TEMPERATURES DURING HEATING

One reason for employing an "instantaneous" method of heating was to separate the effect of the temperature of heating from that of time. The viscosities of the samples (after cooling to 25°C.) are plotted against the temperatures reached by the egg in passing through the heat exchanger.

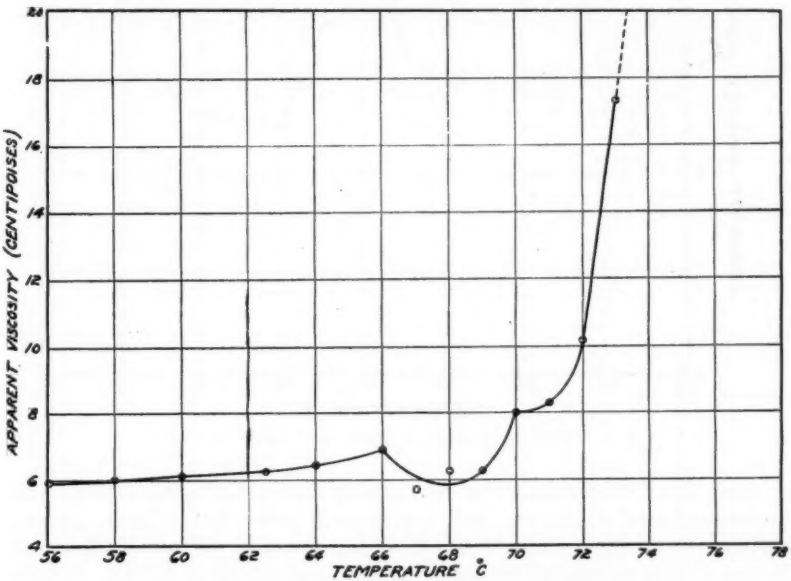

FIG. 4. Effect of heating on viscosity of liquid whole egg.

Whole Egg: The viscosity for whole egg increases slowly, but regularly, with rises in temperature from 56 to 66°C.(132.8 to 150.8°F.) (Fig. 4). A drop in viscosity was observed at 67, 68, and 69°C.(152.6, 154.4, and 156.2°F.). A considerable amount of precipitate was centrifuged out of the samples which showed this drop in viscosity. At 68°C. the viscosity began to increase again, continuing until coagulation set in just above 73°C.(163.4°F.) (as indicated by the dotted lines).

Egg White: The viscosity of egg white does not change with heating (within the time limits studied) until it reaches 62.5°C. (Fig. 5). Above 62.5°C. the effects are extremely irregular, but these irregularities coincide with the changes in appearance discussed above. The viscosity reaches

maxima at 63 and 66°C. No turbidity was observed in the liquid white at these two temperatures, but the material was of a very gelatinous nature. At the points of low viscosity there was a clean-cut separation of the gel flocs, thus allowing for their removal during centrifuging.

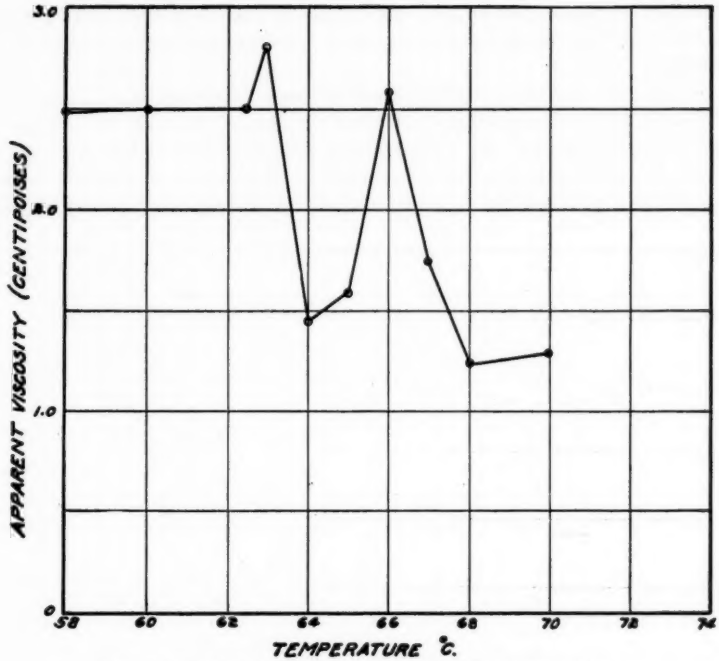

FIG. 5. Effect of heating on viscosity of liquid egg white.

Egg Yolk: The viscosity of native yolk containing 49 to 49.5 per cent water was found to be about 800 centipoises at 25°C. This value is approximately one hundred times as great as the viscosity of liquid whole egg. Moran (1935) calculated that at 18 to 19°C.(64.4 to 66.2°F.) the viscosity of fresh yolk with a water content of 47 to 47.7 per cent was approximately 2,500 centipoises. Comparisons of Moran's value with those obtained in this study reveal some discrepancies which can be explained as due to the temperature at which viscosity was measured and the water content of the yolk sample used. Moran found that the viscosity of yolk is a function of the water content. From his illustration of the effect of water content on the time of outflow, it was found that an increase in water content from 47.7 to 49.5 per cent corresponds to an increase of about 54 per cent in the outflow time (decrease in viscosity). Thus, a yolk sample of 49.5 per cent moisture would have a viscosity of about 1,150 centipoises. The difference in the measurement of temperature and the limitations of the method used in the two cases probably account for the rest of the discrepancies.

The viscosity of egg yolk as it is affected by the temperature of heating is shown (Fig. 6). As in whole egg and in egg white, the effect of heating is to increase the viscosity of the yolk, and as the heating temperature is raised the viscosity continues to rise without interruption until at 70°C. (158°F.) the sample coagulates in the heat exchanger (as indicated by the dotted lines).

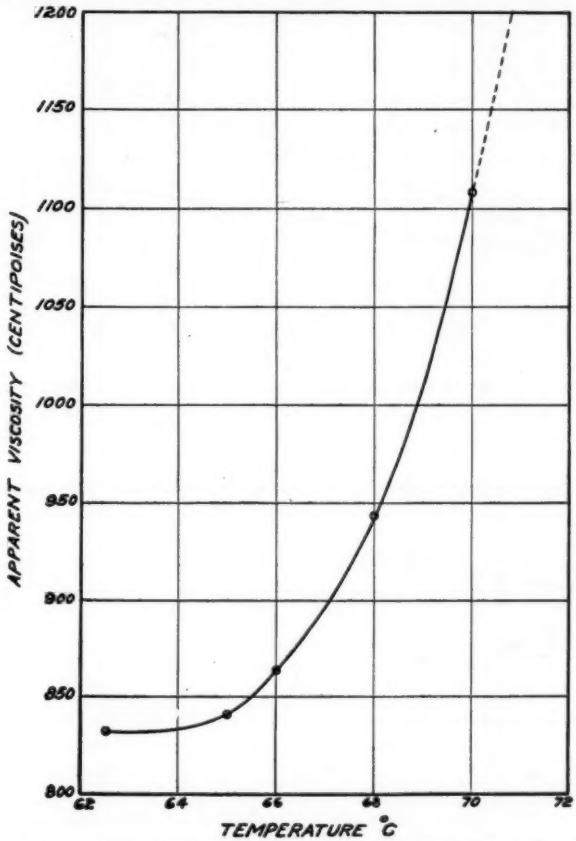

FIG. 6. Effect of heating on viscosity of liquid egg yolk.

EFFECTS OF TIME DURING HEATING

Whole Egg: The effect of time of heating on the viscosity of whole egg (Fig. 7) indicates that the increase is linear with time for the temperature range studied. The slopes of the lines indicate the greatly accelerated rate of change as the temperature rises. There was no change in viscosity on holding at 58°C.(136.4°F.) or below (within the time limits studied).

Egg White: The effect of time of heating on the viscosity of egg white (Fig. 8) shows that no change in viscosity was detected at or below 58°C. The change is quite slow at 60°C.(140°F.) but increases perceptibly with

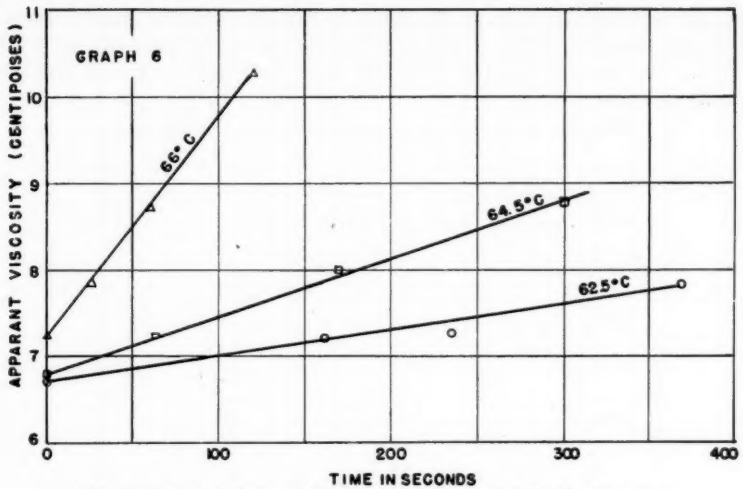

FIG. 7. Effect of time of heating on viscosity of liquid whole egg.

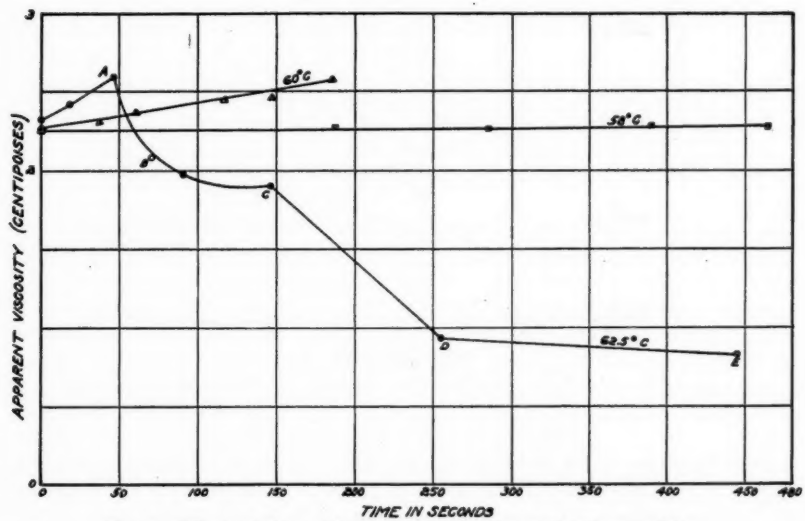

FIG. 8. Effect of time of heating on viscosity of liquid egg white.

time. The maximum viscosity is reached at a heating time of 45 seconds at 62.5°C., and with further holding the viscosity showed erratic changes. As described earlier, whites show changes in turbidity, flocculation, and coagulation during holding at this temperature; these stages are very well indicated (Fig. 8). Point *A* corresponds to the time at which turbidity first becomes evident; flocs of gelatinous material were also observed. At

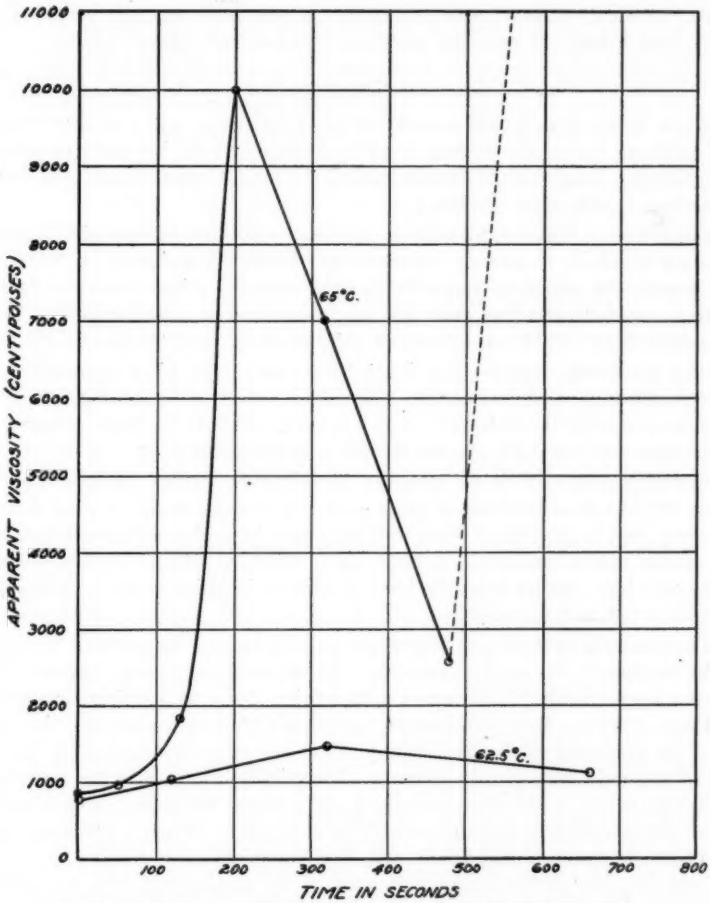

FIG. 9. Effect of time of heating on viscosity of liquid egg yolk.

B there was an increase in the volume of the flocculent material to the extent that it occupied almost the whole volume of the liquid, while at *C* the flocculent material became slightly opaque and started to separate into segments. At Point *D* the coagulum formed into particles similar to swollen rice grains and the supernatant liquid was decidedly more fluid; at Point *E* the coagulum had a slightly firmer consistency.

Egg Yolk: The changes in viscosity of liquid egg yolk with time of heating at 62.5 and 65° C. are shown (Fig. 9). At 65°C. the viscosity of liquid yolk increased with the time held until a maximum was reached; further holding at this temperature resulted in a sharp decrease in viscosity up to approximately 475 seconds. Almost immediately thereafter the rate of flow through the exchanger became very slow and coagulation set in. It will be noted that the rate of change in viscosity was much greater at 65°C. than it was at 62.5°C., where little change occurred. At 58°C., and below, no changes were observed to have taken place.

DISCUSSION

While it was hoped that a study of the appearance and viscosity changes in liquid-egg products during heat treatment would be instrumental in studying the progress of denaturation, it is not surprising that rather anomalous results were obtained.

There are at least eight heat-denaturable proteins in egg white; among them are albumin (1 and 2), conalbumin (1 and 2), globulin (1, 2, and 3), and mucin. In addition, the yolk contains several others, namely, lecithovitelline, cephalo-vitelline, and livetin, according to Cruickshank (1941), Jukes and Kay (1932), and Longsworth, Cannon, and MacInnes (1940).

Our knowledge concerning the heat-denaturation phenomenon for the various proteins is very limited. Even if we knew a great deal about the pure components, the behavior of the natural mixture in yolks, whites, and whole eggs could not be predicted with complete certainty.

Viscosity appears to be a fairly satisfactory means of following the course of heat denaturation in some pure-protein solutions. It now appears, however, that it is only of very limited value in such complex materials as egg whites and yolks. It is interesting to note that the viscosity behavior of liquid whole egg undergoing heat treatment is quite normal. The reason for this is not as yet apparent.

The peculiar behavior of egg whites, when heated, appears to be related to the change in the mucin fraction. This same phenomenon appears to be playing a part in the stabilization of the thick white by heating, as reported by Funk (1943). It is well known that mucin is responsible for the thick, jelly-like property of the firm white. Heat seems to bring about a contraction of the gel. Because the density of mucin is lower than the remainder of the egg white, it shrinks eventually and reaches the point during heating where the mucin-rich fraction centrifuges readily. When yolks and whites are mixed to make liquid whole egg, the phenomenon observed above does not occur. This difference may be caused by changes in pH, electrolytes (kind and amount), protein concentrations, etc., occasioned by mixing the two portions of the egg content together.

The very peculiar results obtained during the heating of yolk at 65°C. (where the viscosity rose sharply, suddenly fell, and then almost instantly coagulated) is difficult to explain. It is conceivable that the fat emulsion broke, giving rise to a very much lowered viscosity. (It is known that as the state of subdivision increases in emulsions, there is an increase in the viscosity.) To explain the present phenomenon, the effect of breaking the

emulsion would have to be very much greater than that caused by the denaturation of the yolk proteins. No direct evidence is offered for this explanation.

The practical importance of the various changes observed during heat treatment remains to be ascertained by studies on the specific effects of heating on the functional properties. Some of the more important of these properties are leavening power (sponge and angel cakes), emulsifying power (salad dressing), and thickening power (custards).

The present studies serve only to orient the work for future study leading more directly toward these practical problems. The next paper in this series will cover these aspects.

SUMMARY

Denaturation of liquid-egg products is a function of the temperature and time of heating. Viscosity, as determined by a capillary viscometer, was used as a criterion of the effect of heat treatments on denaturation. It was found to be a sensitive index.

Temperature of Heating: The region of denaturation for liquid whole egg, as determined viscometrically, occurs within the temperature range of 56 to 66°C. At temperatures above this range, fractional precipitation of the proteins and a general breakdown of the system are indicated by an irregular viscosity-temperature relationship. Above 73°C. whole egg coagulates almost instantaneously.

In liquid egg white denaturation occurs in the temperature range of 58 to 62°C. As in the case of the whole egg, the irregular character of the viscosity-temperature relationship above 62.5°C. indicates the region at which fractional coagulation of the proteins occurs.

The denaturation of liquid egg yolk within the temperature range of 62.5 to 70°C. is characterized by an increasing rate as the temperature rises. Above 70°C. coagulation occurs almost instantaneously.

Time of Heating: Viscosity in liquid whole egg increases as a linear function of the time of heating, with the rate being more rapid at the higher temperatures.

A linear viscosity-time relationship is shown by liquid egg white at 58 and 60°C. However, the region of denaturation at 62.5°C. is very short. Beyond a certain period of heating (46 seconds) at 62.5°C. the fractional coagulation of the proteins is indicated by a drop in viscosity of the liquid white.

At 62.5°C. viscosity of liquid egg yolk is a linear function of the time of heating. Beyond a certain period of heating (300 seconds) a drop in viscosity is observed. At 65°C. viscosity was not linear with time; it increased very rapidly until a maximum viscosity at 200 seconds was reached. A sharp drop in viscosity beyond this point was observed before coagulation occurred.

ACKNOWLEDGMENT

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SOME COOKING QUALITIES OF HOMOGENIZED MILK. II. WHITE SAUCES[1]

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Despite the increase in the demand for and consumption of homogenized milk, information on its cooking qualities is limited. Hollender and Weckel (1940, 1941) noted that, when homogenized milk was used in cooking oatmeal and wheat cereals, its stability was less than that of unhomogenized milk. The addition of sodium chloride affected the stability of the milk, delaying the coagulation. In escalloped potatoes the homogenized milk coagulated sooner than unhomogenized milk, but the extent of the defect was dependent on the quality and chemical composition of the potato. The addition of various salts, such as sodium citrate, sodium carbonate, and sodium phosphate, did not always prevent curdling of the milk and thus could not be recommended indiscriminately for the correction of the instability of homogenized milk in potato cookery. These workers observed also that the curd tension of egg custard prepared from homogenized milk was lower, the serum separation greater, and more critical temperature-time range existed than when unhomogenized milk was used.

Carr and Trout (1941) found that the cooking time for baked custards made with homogenized milk was from 15 to 20 minutes longer than for custards made with similar milk not homogenized. However, baked custards made with homogenized milk were more firm than those made with unhomogenized milk and less syneresis occurred. Soft custards made with homogenized milk were found to be more viscous than those made with unhomogenized milk.

Inquiries have been received concerning the curdling of gravies made with homogenized milk which suggest the need for specific information concerning the qualities of homogenized milk in this type of cookery. The data reported herein concern the use of homogenized milk in white sauces.

EXPERIMENTAL PROCEDURE

White sauces, rather than pan gravies, were chosen since cooking temperatures could be controlled. These were made in small aluminum double boilers, using for each lot 244 grams of milk with various portions of flour, salt, and fat. Part of the sauces were made with fats commonly used for making gravies, such as bacon, beef, sausage, and mixed drippings, but butterfat was the reference fat chosen. Milk homogenized at 2,500 pounds pressure was used for all experiments except one series designed to study the influence of homogenization. In this case, milk was prepared at pressures ranging from 500 to 4,500 pounds. The sauces were cooked for five minutes after the temperature reached 90°C.(194°F.). The effects of various methods of mixing and manipulation were studied.

[1] Michigan Agricultural Experiment Station, Journal Article No. 764 N.S.

Consistency of the sauces was determined by the line-spread test of Grawemeyer and Pfund (1943). The sauces were judged subjectively by a group of trained people who had no knowledge of the identity of the samples. In determining the percentage of free fat in the sauces 10-gram samples were weighed into calibrated Pyrex centrifuge tubes, then were centrifuged at 4,000 r.p.m. for 10 minutes in a No. 1, Type 5 B, International centrifuge. Sudan III was used to stain the fat in the samples that were microphotographed.

RESULTS

Sauces made with homogenized milk, instead of being smooth and homogeneous, often have a separated, curdled appearance. True curdling results when the milk protein precipitates, giving a separation of curd and whey; whereas, in these white sauces separation of fat was seen as fat droplets throughout the mixture and on the surface. The appearance was that of curdling; however, the two conditions are distinct. Evidently, the difficulty encountered in making white sauces with homogenized milk is that of fat separation.

Influence of Proportion of Fat to Flour: Preliminary trials suggested that the fat separation in white sauces made from homogenized milk might be the result of unemulsified fat exuding to the surface (Fig. 1). When the fat was omitted, smooth sauces resulted regardless of the type of milk used. As the amount of fat added was increased, there was a corresponding increase in fat separation, as shown by the centrifuging technique (Fig. 2). This method of determination is not distinctive, however, since the amount of separation from sauces made with unhomogenized milk was similar. Sauces made with homogenized milk and five grams of fat showed no evidence of fat separation (Figs. 1, 10a, 10b, and 10c); six to 12 grams of fat yielded fine droplets of fat; whereas 13 grams or more of fat in the sauces resulted in proportionately larger fat droplets. When an excess of fat (28 grams) in proportion to the amount of flour (10.4 grams) was used, a sauce in which the fat masses appeared to "stand out" on the surface resulted. On the other hand, sauces made with unhomogenized milk appeared smooth until an excessive amount of fat (35 grams to 10.4 grams flour) was used, although data (Fig. 10a) demonstrate that the fat was present in finely divided particles rather than in combination in the sauce. When these proportions of fat to flour were used, a sauce was obtained, the appearance of which compared favorably with that made with homogenized milk in which only 10 grams of fat and 10.4 grams of flour were used. Thus, when making white sauces with homogenized milk, accuracy of measurement of ingredients becomes more essential than when using unhomogenized milk, inasmuch as the "factor of safety" as to quantity of fat is narrower. Approximately three and one half times more fat can be used in making white sauces without noticeable fat separation when using unhomogenized than when using homogenized milk.

Judges noted little difference between samples as far as fat separation was concerned until more than 11 grams of fat were used (Table 1). However, when a sauce was made with less than 11 grams of fat the judges criticized it as being pasty and unpalatable. The judges' average opin-

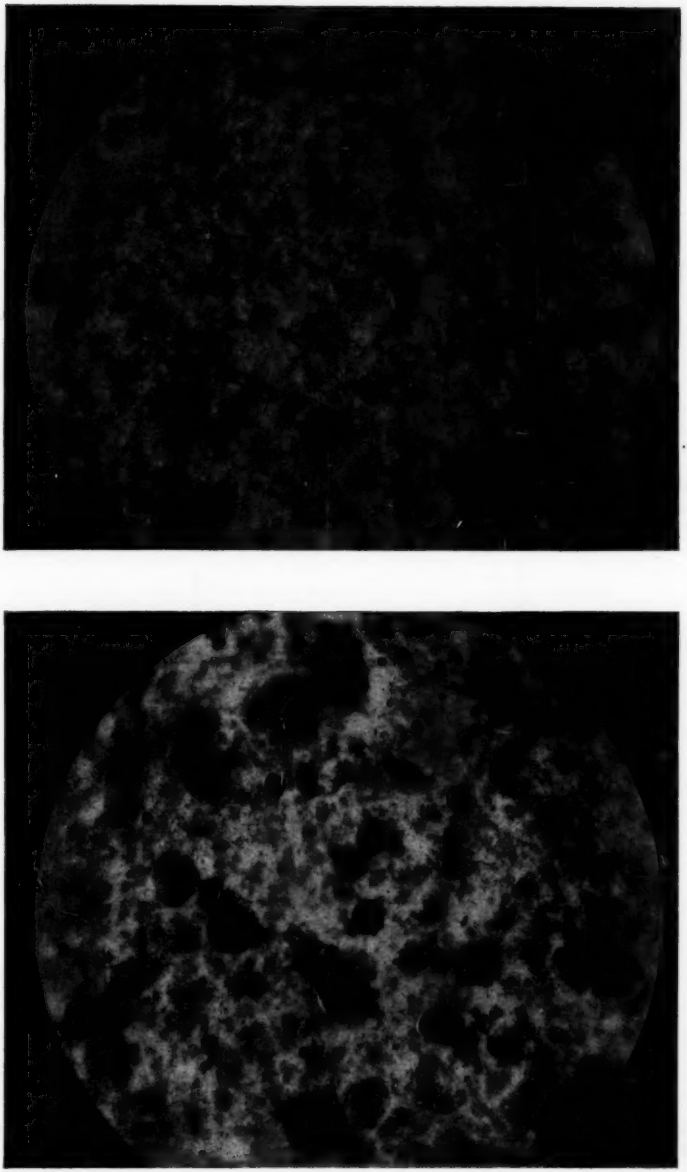


FIG. 1. Photomicrographs showing the surface of white sauces made with homogenized milk, (a) without and (b) with added 10.4 grams fat (× 48).

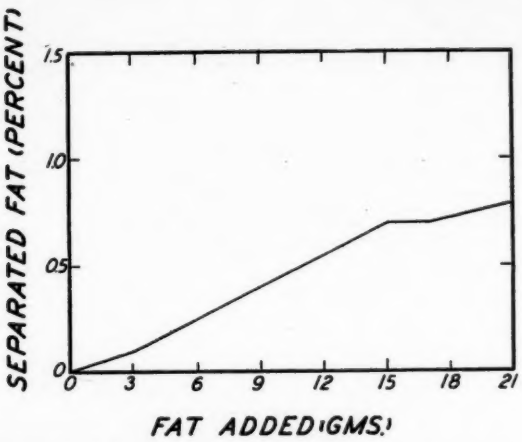


FIG. 2. Percentage of fat separating in white sauces made with homogenized milk and increasing amounts of fat, centrifuged at 4,000 r.p.m. for 10 minutes.

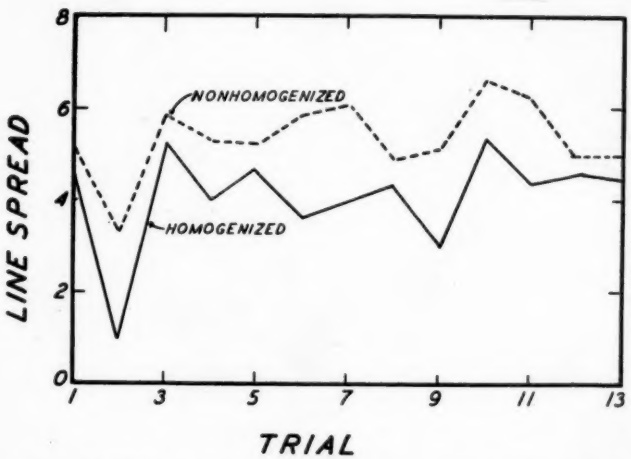


FIG. 3. Viscosity of white sauces made with unhomogenized and with homogenized milk as shown by line-spread.

TABLE 1

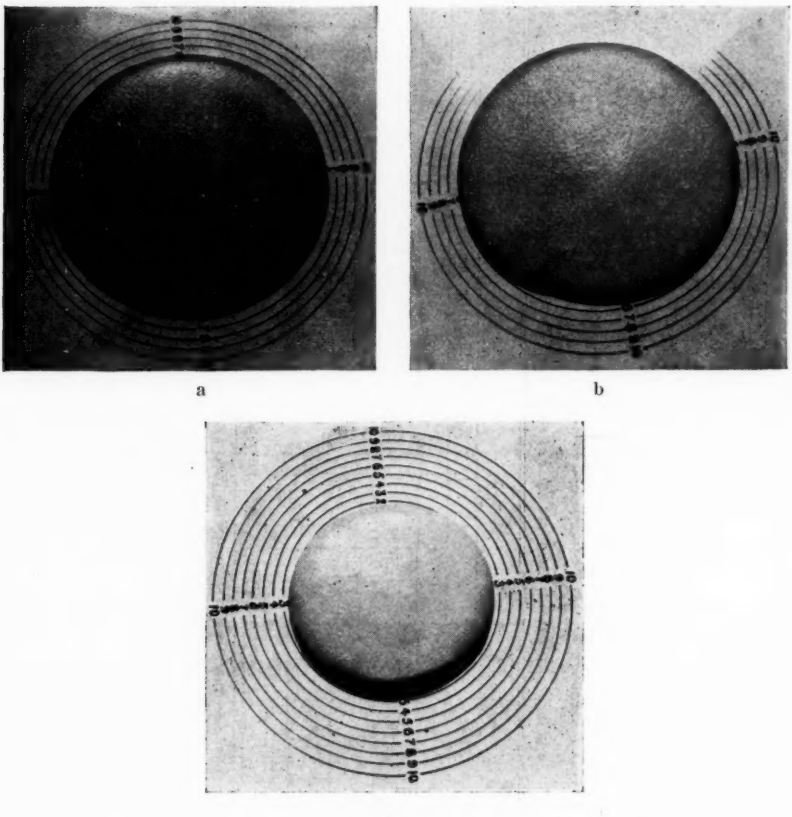
Influence of Various Amounts of Fat on Some Characteristics of White Sauces

Quality factor	Homogenized milk									Unhomogenized milk
	Butterfat (gm.)									Butterfat (gm.)
	0	3	7	9	11	13	15	17	21	21
Texture (degree of fat separation)	3.0[1]	3.0	2.9	3.0	2.7	2.3	1.2	1.6	1.1	2.2
Small, "free" fat droplets	3.0	3.0	2.9	3.0	2.7	2.1	1.3	1.3	1.1	2.1
Freedom from curdling	3.0	3.0	3.0	3.0	3.0	2.9	2.1	2.6	2.1	2.9
Palatability	1.3	1.9	1.9	2.1	2.3	2.5	2.6	2.5	2.8	2.5

[1] These are average scores of five judges. In each case 1 represents the least desirable and 3 the most desirable product with respect to the quality being scored.

ions agreed closely in respect to the texture, absence of "free" fat, and curdling.

Homogenized milk sauces were more viscous than unhomogenized milk sauces of similar composition, as demonstrated by the line-spread test (Fig. 3) and by the general appearance of the sauces (Fig. 5). For sauces of equivalent viscosity those made with homogenized milk require only three fourths as much thickening agent as the ones made of unhomogenized milk.



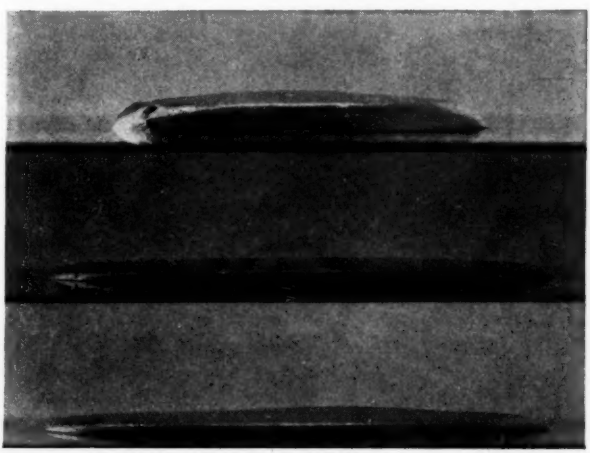
a b c

FIG. 4. Line-spread and appearance of white sauces made (a) from unhomogenized milk, (b) from homogenized milk, and (c) from milk into which the sauce fat was homogenized.

Since, from the evidence presented above, it appeared that the fat added did not combine in white sauces when blended during cooking, an equivalent amount of butterfat colored with Sudan III was homogenized into the milk to ascertain if sauces made from this homogenized mixture would show fat separation. Sauces made with homogenized-fat fortified milk were smooth, creamy, very thick, and uniform throughout (Fig. 4).

a b c

FIG. 5. Coating on spoons of white sauces made (a) with unhomogenized milk, (b) with homogenized milk, and (c) with milk in which the sauce fat was homogenized into the milk.



a

b

c

FIG. 6. Fluidity of white sauces made (a) from milk in which the sauce fat was homogenized into the milk, (b) with regular homogenized milk, and (c) with unhomogenized milk.

The average line-spread was 2.016 as compared with 4.75 for the sauce made by the regular method using plain homogenized milk. The relative smoothness, viscosity, and appearance of the sauces made with unhomogenized, homogenized, and homogenized-fat fortified milk may be noted (Figs. 4, 5, 6).

Influence of Kind of Fat: Sauces were made using bacon fat, beef fat, sausage fat, and mixed drippings as well as butterfat. Visible fat separation was noted in all the sauces made from these fats when homogenized milk was used. The size of the droplets of fat varied somewhat, but, in general, those from the sauces made with fat other than butterfat were slightly finer than those made with butterfat. The viscosity of the sauces, as shown by the line-spread, varied slightly according to the source of fat but the variation seemed to be of little significance (Table 2).

TABLE 2

Viscosity of White Sauces Made With Homogenized Milk and Various Fats

Source of fat	Line-spread
Bacon	4.7
Beef	4.0
Butter	4.4
Mixed drippings	3.7
Sausage	3.6

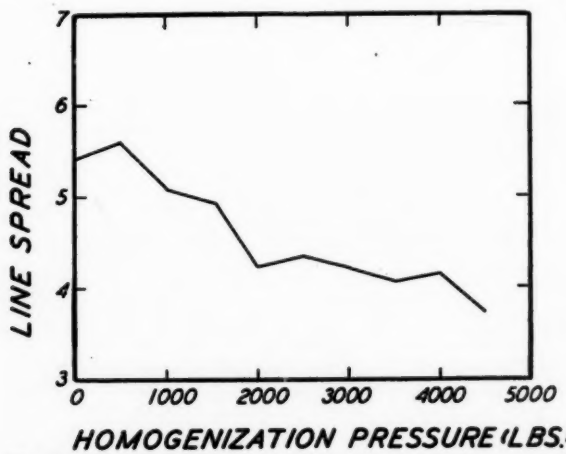


FIG. 7. Viscosity of white sauces made with milk homogenized at various pressures as shown by line-spread.

Influence of Salt: The influence of certain salts, particularly sodium chloride, was studied. As the amount of sodium chloride in the mixture was increased the degree of fat separation increased also. Likewise, salt added at the beginning of cooking caused greater fat separation than when added at the end of cooking. That salt was not the cause but does have a

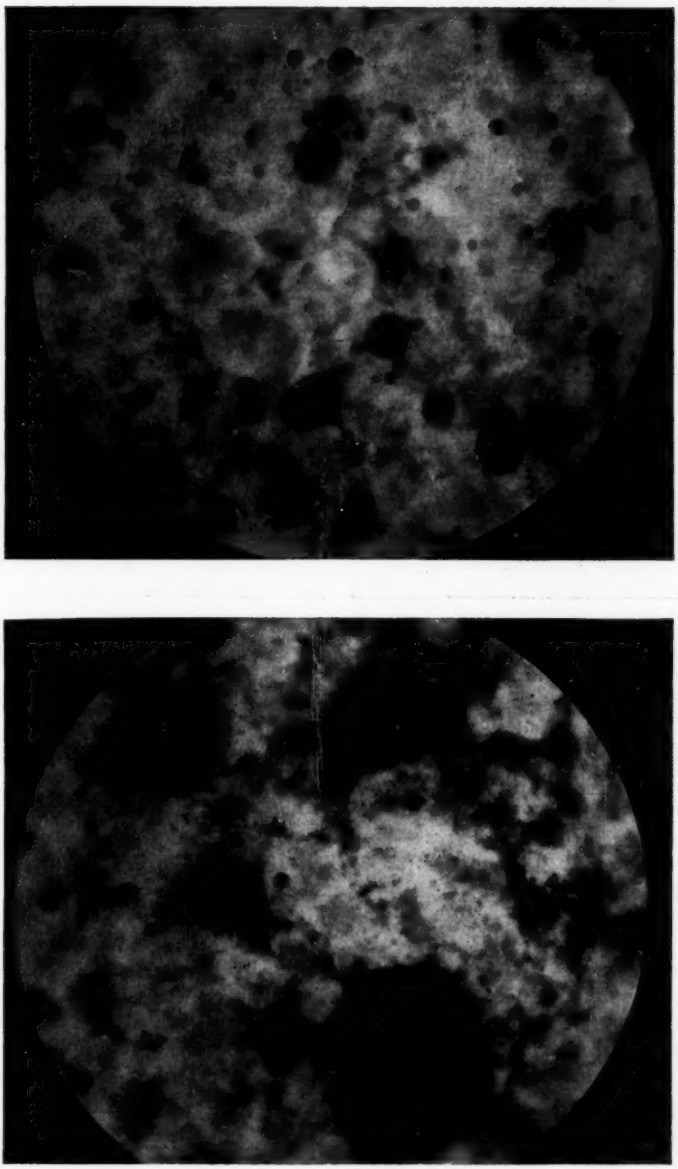
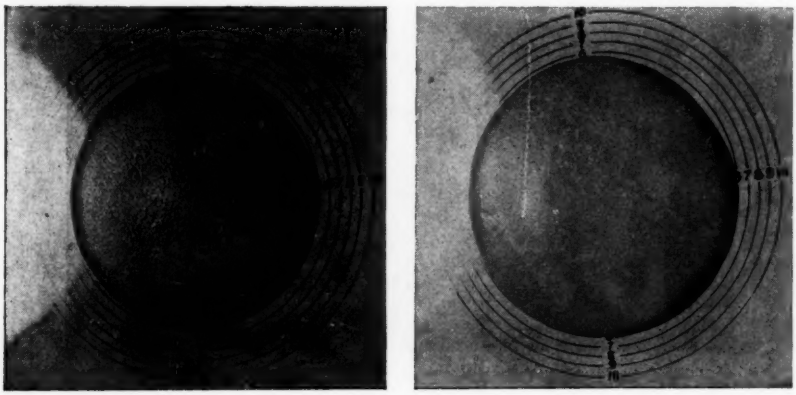


FIG. 8. Size of the fat droplets in sauces made from homogenized milk (a) homogenized at 1,000 pounds pressure and (b) at 3,000 pounds pressure (× 48).

contributing influence to separation, was verified by making a series of sauces with washed butter as the sample of fat.

The addition of half-molar solutions of calcium chloride, disodium phosphate, sodium bicarbonate, or sodium citrate failed to prevent fat separation. The order of the salts in relation to increasing degree of fat separation was as follows: disodium phosphate, sodium bicarbonate, sodium citrate, and calcium chloride. Calcium chloride caused both "oiling off" of the sauce and precipitation of the casein.



a b

FIG. 9. Macroscopic appearance of white sauces made from homogenized milk (a) before and (b) after beating.

When portions of the sauces to which the various salts were added were centrifuged, little variation was noted in the amounts of free fat obtained, thus indicating that, even in the so-called smooth sauces, the fat actually is present in an uncombined state but in smaller, dispersed droplets.

Influence of Various Pressures of Homogenization: White sauces were made from milk homogenized at 0, 500, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, and 4,500 pounds per square inch. As the pressure of homogenization increased the sauces became more viscous, as shown by the reduced line-spreads (Fig. 7). The extent of separation of added fat was greater with each increase in pressure, as evidenced by larger droplets of free fat in the completed sauce (Fig. 8). Particularly were the droplets noticeably larger when the sauce was made from milk homogenized at 2,000 pounds pressure and above.

Fat separation in white sauces was not noted when they were made without added fat even though high-pressure homogenized milk was used. This observation was further substantiated with cornstarch puddings made with homogenized milk which, although smoother and more viscous than those made with unhomogenized milk, failed to show any fat separation.

Effect of Beating: Since the added fat seemed to be the constituent which separated in the cooked sauce, and fat may be combined with other constituents by beating, it seemed desirable to ascertain the effect of

beating the sauce both after cooking and during cooking. This was done by means of a hand rotary beater. All the sauces when beaten for two minutes, including those from unhomogenized milk, had an apparent smoothness that was not present in the unbeaten sauces (Table 3, Fig. 9).

TABLE 3

Influence of Beating Upon Some Characteristics of White Sauces

Quality factor	Sauces made from			
	Unhomogenized milk		Homogenized milk	
	Not beaten	Beaten	Not beaten	Beaten
Texture (degree of fat separation)	2.2[1]	3.0	1.0	2.9
Size of fat droplets	2.0	3.0	1.0	2.9
Freedom from curdling	2.3	3.0	1.5	3.0
Palatability	2.5	2.9	2.4	3.0

[1] These are average scores of seven judges. In each case 1 represents the least desirable and 3 the most desirable product with respect to the quality being scored.

The beaten sauces made with homogenized milk not only remained smooth upon standing but retained this smoothness when cooled and reheated. The sauces that were beaten for two minutes after removal from the fire appeared slightly smoother than those which were beaten while cooking. However, photomicrographs showed merely dispersion of fat globules which compared favorably in size with those of the sauces made from unhomogenized milk (Fig. 10). Likewise, there was no difference in the amount of fat separated out from the beaten and unbeaten sauce when samples were centrifuged.

A practical technique for producing an acceptable sauce made with homogenized milk is to bring about a finer dispersion of the fat globules by means of beating.

DISCUSSION

From the above experiments with sauces made from homogenized milk it appears that the fat used in making the sauces did not combine with the fat of the milk, or at least failed to enter into a state of emulsion comparable with that obtained when the milk was not homogenized. A probable explanation for this behavior might be found in the changed surface area of the fat globules as the result of homogenization. Wiegner (1914) calculated from viscosity measurements that the fat globule of the unhomogenized milk absorbed about two per cent. Accordingly, the surfaces of the fat globules of homogenized milk might be more completely covered than those of unhomogenized milk despite the marked increase in surface area of the fat globules as a result of homogenization. Consequently, there would be less opportunity for fat-to-fat contact between the added fat and the fat of homogenized milk when making sauces of the latter, thus resulting in "free" fat and hence fat separation in the finished product. When the added fat is homogenized with the milk it is so finely dispersed that sauces made from it are smooth, showing no fat separation. This leads one to believe that the cause of fat separation in sauces made from homogenized milk is, in large part, the result of the inability of the added fat to unite with the fat of the milk.

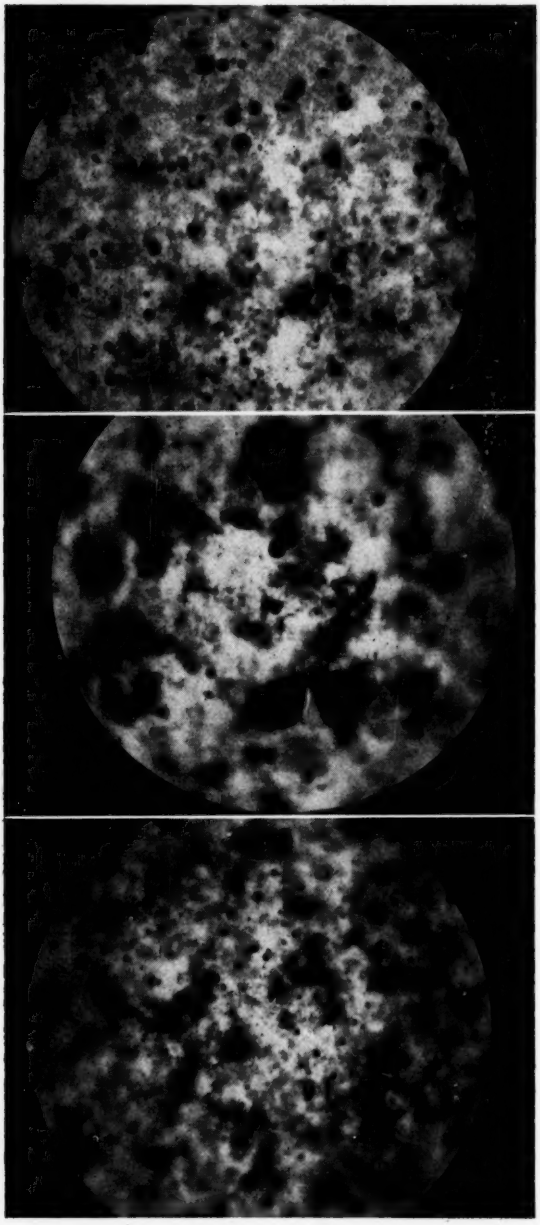


FIG. 10. Photomicrograph of white sauces made (a) from unhomogenized milk, (b) from homogenized milk, and (c) from homogenized milk and beaten two minutes.

Sauces made with homogenized milk were thicker than those made with unhomogenized milk; approximately one fourth less flour was needed for a sauce of comparable viscosity. This agrees with the work of Carr and Trout (1942), who noted increased viscosity of custards made with homogenized milk.

SUMMARY

White sauces were used to study the effect of homogenization on the cooking properties of milk. Sauces made with homogenized milk showed a separation of fat in contrast with those made with unhomogenized milk which appeared smooth. As the amount of added fat was increased, the size of the fat droplets and apparent amount of separation became greater.

Increased amounts of salt caused the formation of larger fat droplets. The fat droplets were not as large when salt was added at the end of the cooking period.

Increased pressures of homogenization increased the size and apparent separation of the fat globules. The viscosity of the sauces became increasingly greater as the homogenization pressure used in preparation of the milk was increased.

Beating sauces made with homogenized milk with a rotary beater produced a product smoother and superior in flavor to those made with unhomogenized milk.

ACKNOWLEDGMENT

Credit is due Dr. Pauline C. Paul, section of Home Economics, for the taking of the photomicrographs, and to Mr. E. N. Huby, college photographer, for the other pictures shown herein.

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PREVENTION OF MOLD IN KETTLED BLUEBERRIES IN NO. 10 CANS

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(Received for publication, June 28, 1945)

Visible mold colonies in the headspace of certain canned blueberries was first observed during the 1938 pack. Studies immediately initiated with a view to eradicating the defect have been continued in the laboratory and in the field. No complaints of mold after the product has entered trade channels have been raised. The occurrence of this particular mold in blueberries and its unusual heat resistance, however, was reported by Williams, Cameron, and Williams (1941). It is believed, therefore, that possible methods of control available to blueberry packers as a result of the study of the problem by this laboratory will be of interest.

As previously reported by Williams, Cameron, and Williams (1941), the mold isolated from kettle-pack blueberries canned in No. 10 size cans was found to be a hitherto undescribed strain of *Penicillium* capable of growth in relatively high vacuums until the small percentage of oxygen remaining in such rarefied atmosphere is completely exhausted. The growth shows a variety of colors and occurs usually as round colonies up to one inch or more in diameter on the undisturbed surface of the product after the cans have stood for a few days. Positive cans characteristically have good vacuums, around 15 inches, and headspaces in the normal range of $^{16}/_{32}$ to $^{20}/_{32}$ of an inch.

Preliminary studies centered about the heat resistance of the various forms of the mold, and the results of thermal death-time tests run in this laboratory have been comparable to those previously reported. The fact that the mold sclerotia may survive a temperature of 85°C.(185°F.) for a few hours and 87.8°C.(190°F.) for about one hour eliminated the commercial possibility of destroying such forms by heat. Such temperatures applied for the time required would seriously affect the quality of the product. Further, such temperatures are not necessary to destroy the common molds, yeasts, and nonsporeforming, acid-tolerant bacteria capable of development at the normal pH (3.4 to 3.7) of blueberries, which are the usual causes of spoilage in this product. Thus, it became apparent that control measures depended either upon the entire elimination of air from the can or upon keeping the product free of the mold sclerotia.

OXYGEN REQUIREMENTS OF THE ORGANISM

To determine more accurately the oxygen requirements of this mold, tube cultures were exposed to varying amounts of oxygen mixed with nitrogen or carbon dioxide. In addition, mixtures of carbon dioxide and nitrogen with oxygen were tried.

The procedure used for the oxygen requirement study involved sterilization of juice from canned blueberries in 20 x 150 ml. test tubes, and after cooling, inoculation with the stock *Penicillium* sclerotia suspension.

The tubes were then swept with the desired gas mixture previously prepared in a gas burette by bubbling through the juice. The glass inlet tubes through the two-hole rubber stopper were then sealed off and the stopper dipped in paraffin. Observation for growth was made during incubation at room temperature. The results obtained are shown (Table 1).

TABLE 1

Mold Growth in Blueberry Juice in Presence of Various Gases and Gas Mixtures

Oxygen	Nitrogen—growth[1]		Carbon dioxide—growth[1]	
pct.	*pct.*		*pct.*	
20	80	++++	80	++——
10	90	++++	90	—————
5	95	++++	95	—————
1	99	++++	99	—————
0.5	99.5	+++—	99.5	—————
0	100	—————	100	—————

[1]+ = Visible mold growth per culture tube.

As will be noted from this table, growth of this organism can occur with as little as 0.5 per cent oxygen in nitrogen present. However, growth was inhibited in the presence of as much as 10 per cent oxygen in carbon dioxide. This is in agreement with the work of Golding (1937, 1940a, 1940b) on the effect of nitrogen and carbon dioxide upon the growth of *Penicillium roquefortii.* In other studies made in this laboratory, growth of the blueberry penicillium was found to be inhibited by a mixture of 10 per cent oxygen, 45 per cent carbon dioxide, and 45 per cent nitrogen.

EFFECTS OF LOW-OXYGEN CAN CLOSURE ON GROWTH

Results of the study of the oxygen requirement of the mold suggested that control embodying oxygen starvation would necessitate either essentially complete air exclusion from the sealed can or substantial dilution of any enclosed air with carbon dioxide. Possible beneficial application of a type of can-sealing machine which sweeps air from the can headspace at the time of closure with an inert gas was immediately considered. Because of the high vacuum created in No. 10 kettled blueberries by the very hot filling temperature, the use of carbon dioxide at closure was believed impractical. The additional vacuum resulting from solution of carbon dioxide from the headspace would undoubtedly cause excessive collapse of the can walls or "panelling" of the container. Experimental work was, therefore, undertaken to study nitrogen gas closure under commercial operating conditions.

Commercial pack No. 10 cans of blueberries were inoculated with the sclerotia of the mold and closed under 6¼ inches water pressure-nitrogen flow. The development of the mold was prevented almost entirely by this procedure under selected conditions, but the test packs showed the necessity of eliminating air entrapped mechanically in the kettled berries during the churning and splashing of rapid hand filling of the can as practiced commercially. In commercial practice, the cooked berries are dipped by hand from the kettle into the cans, two dipperfuls to the No. 10

can, and the cans are immediately conveyed on a short track to the closing machine. Air incorporated into the product thus has scant opportunity to rise to the surface through the berries and escape. As a result (Table 2) mold growth occurred in many of the nitrogen-closed test cans with various can headspaces up to one-half inch when sealed immediately after hand filling, but with one exception (Code 7G1) did not occur in duplicate lots held 10 minutes prior to closure.

From these results, it would appear that can closure under nitrogen gas would prevent visible growth of the mold if used in conjunction with methods of eliminating air from the product. Either nonaerating mechanical filling or elapse of sufficient time after hand filling to permit the incorporated air to be expelled from the hot product, would be probable means of accomplishing this. Under continuous production, the necessary holding time for the open can could be provided by conveying the cans on a long track to the closing machine as the berries hold their heat well. A safety factor against understerilization from inadequate closing temperature would be provided, however, if the holding time were obtained during passage of the container through an exhaust box.

As previously stated, our test-tube experiments also indicated that mold growth is inhibited in as much as 10 per cent oxygen mixed with 45 per cent of both carbon dioxide and nitrogen. Thus, it is possible that the use of a mixture of some carbon dioxide with nitrogen could be worked out for commercial operation without excessive panelling, which would not require the complete elimination of air trapped in the product through use of a holding period.

CONTROL BY ELIMINATION OF INFECTION

To locate the source of contamination of blueberries with heat-resistant mold forms, portable bacteriological laboratory equipment was taken into the field during the 1940 season. In all, 76 samples of the raw, incoming berries, representative of lots purchased from neighboring localities for canning, were examined. Heat-resistant mold forms were subsequently isolated from 11 (14 per cent) of the samples, five of which were strains of a *Penicillium* and similar to the spoilage organism originally isolated. The positive samples were not limited to any particular barrens but represented samples from widely scattered regions. In view of the positive soil samples reported by Williams, Cameron, and Williams (1941), it seems probable that the original source of the organism is in the soil, as those authors reported.

Shortly before the initial pack of the season, samples were also collected for examination from the equipment at one plant known to have experienced previous difficulties with mold growth in canned samples. However, only one positive sample, collected from a field winnowing machine, was obtained from 28 samples representative of 13 pieces of equipment in contact with the berries during the previous season. This would indicate that mold forms do not survive in large numbers from season to season in the plant.

Blueberry samples were collected periodically in the cannery from various points considered possible points of build-up of contamination. As would be expected from the occurrence of the mold on the raw, incoming

TABLE 2

Mold Growth in Inoculated No. 10 Cans of Blueberries Closed Under Nitrogen

Code	Closing hdsp.	Description	Total cans packed	Cans showing visible mold growth			
				Extent top surface covered			
				None	Less than ¼	¼ to ¾	¾ to all
	in.						
7G1X	¼	Cans closed quickly under nitrogen	6	6[1]	0	0	0
7G1	¼	Cans held 10 min.—closed under nitrogen	6	6[2]	0	0	0
7-1	¼	Control—not gassed	2	0	0	2	0
7G2X	⅜	Cans closed quickly under nitrogen	6	4[2]	2	0	0
7G2	⅜	Cans held 10 min.—closed under nitrogen	6	6	0	0	0
7-2	⅜	Control—not gassed	2	0	0	1	1
7G3X	½	Cans closed quickly under nitrogen	6	0	6	0	0
7G3	½	Cans held 10 min.—closed under nitrogen	6	6	0	0	0
7-3	½	Control—not gassed	2	0	0	1	1

[1] Four cans showed slight growth which could only be identified microscopically. [2] One can showed slight growth which could only be identified microscopically.

berries, positive samples were frequently found on the berries prior to washing and cooking. Considerable decrease was found in incidence of mold, however, as a result of thorough washing of the berries and of cleaning of the inspection belts, conveyors, and other equipment after each shutdown.

Any reuse of washing water was shown to be conducive to wider distribution of contamination. In one test, mold developed in eight of 25 cans (32 per cent) packed from berries washed by submersion in recirculated water, but in only three out of 66 cans when fresh water was employed. In another test, mold contamination including all types present on the raw berries was reduced from an estimated 10,000 to approximately 800 molds per ounce of raw berries, by fresh water-submersion washing. Again, the mold contamination decreased from 12,000 to 2,800 molds per ounce of berries in the submersion wash, and dropped to an estimated 580 after an experimental spray wash. Thus, the possibility of further decreasing the mold contamination by combination submersion washing and spray rinsing was demonstrated.

Berries and debris occasionally adhering to and souring on the equipment in inaccessible spots difficult to clean were consistently contaminated with heat-resistant mold forms. It was demonstrated that mold would develop in the can should any of the debris become dislodged into the passing product. Consequently thorough attention to cleaning every portion of the equipment after each shutdown is highly important to control of contamination. Another source of infection was found to be the wooden shop boxes used for holding and transferring berries. Since wood is a consistent offender in contributing spoilage organisms to foods because of its porous nature, metal boxes should be substituted where transfer boxes are required.

PLAIN *VERSUS* ENAMEL CANS

In the factory in which the moldy condition was first observed, growth was reported in certain of the enamel-lined and not in the plain No. 10 cans used for part of the pack. As a result, it was first suspected that the difficulty was in some way associated with the use of the enamel can, and, owing to the low-oxygen requirements of the organism, it was assumed, as explained by Williams, Cameron, and Williams (1941), that the more rapid consumption of residual oxygen in the plain can through oxidation of the exposed metal surface was involved.

With this in mind the possibility of preventing mold growth by using No. 10 plain cans was investigated by inoculated packs in both plain and enameled cans packed under identical commercial conditions. Contrary to previous conclusions, comparable growth occurred in both plain and enamel cans filled to the same headspace. The tests showed definitely that the extent of mold growth in the headspace of No. 10 canned blueberries is not noticeably affected by any difference in rate of oxygen disappearance that may exist between the two types of cans.

SUMMARY

The occurrence of heat-resistant mold forms on raw blueberries and means to prevent subsequent growth in the headspace of the No. 10 canned

product have been studied. Presence of spores of the mold on berries grown in many areas indicates that the contamination of the raw product is quite common. Considerable reduction in infection of the canned product can be effected by careful attention to thorough washing of the fruit and to careful cleaning of inspection belts and equipment in the plant. Complete control by such means alone, however, would appear to be difficult under commercial conditions.

Visible mold growth can be prevented by elimination of oxygen from the can by substituting nitrogen or other inert gas for the air in the headspace. Means must also be provided for exclusion of air mechanically entrapped in the berries or for permitting it to escape before sealing the can with inert gas in the headspace.

Comparable mold growth occurred in both plain and enameled cans and was not noticeably affected by any difference in rate of oxygen disappearance between the two types of cans.

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A REVIEW OF *MICROCOCCUS* ENTEROTOXIN FOOD POISONING

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Prior to 1930, the ability of micrococci to elaborate a substance capable of provoking symptoms of food poisoning in human beings was almost unsuspected. There had been indications earlier that these organisms might be associated with gastrointestinal disturbances, but, in general, their presence in suspected foods was attributed to their ubiquitous distribution.

Denys (1894) was among the earliest of the investigators to suspect micrococci as being responsible for gastrointestinal disturbances and reported an outbreak which resulted from ingesting the meat of a cow that died of puerperal fever. Bacteriological examination of the meat yielded numerous yellowish micrococci, but no organisms of the coliform group. Fresh meat obtained from various butcher shops was examined and it was found that organisms of the coliform group often predominated, but micrococci were rarely present. One death resulted from this outbreak and micrococci were recovered from the spleen of the deceased. Attempts to infect laboratory animals with cultures of these micrococci were further fruitless. In conclusion, Denys stated "the harmful qualities of the meat from the cow that died of puerperal fever are quite probably due to the presence of *Staphylococcus pyogenes.*"

That micrococci were capable of causing meat poisining was accepted by Owen (1907) who commented "other observers have attributed the poisonous character of the meat to the presence of staphylococci . . ." He investigated an outbreak that was due to the consumption of dried beef. Nineteen persons who partook of this meat became ill with symptoms which now would be considered typical of micrococcic food poisoning. Almost a pure culture of white micrococci was obtained from the meat and to them Owen attributed the harmful effects of eating the beef. All efforts to obtain a soluble toxin by filtration of culture through Berkefeld filters were unsuccessful although he reported that laboratory animals were susceptible to inoculations of "beef tea emulsions of growth."

Seven years later, Barber (1914) reported upon the milk of a cow which repeatedly caused intestinal upsets in consumers. He showed that the milk was harmless unless it was allowed to incubate for several hours at room temperature. Human volunteers reacted repeatedly to milk inoculated with the white *Micrococcus* which he isolated and which was allowed to incubate for a few hours. However, kittens, pups, and monkeys were not susceptible to oral administration of milk cultures. This was the first indication that a substance elaborated by the micrococci and not the

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micrococci themselves was responsible for gastrointestinal disturbances. It was also the first case in which the micrococci were isolated in pure culture, inoculated into sterile media, incubated for several hours, and fed to human volunteers who experienced symptoms similar to those originally observed in the victims of the outbreak.

Although this evidence was conclusive it did not receive the recognition it merited, and micrococci were not recognized as causative agents of food poisoning in which they were found, even though no other toxigenic organisms were present. Thus, Nelson (1928), reporting upon an outbreak involving 151 persons, failed to consider the micrococci present in the suspected chocolate cream pie as the cause. The other organisms present, i.e., a *Streptococcus* and an organism of the coliform group, were eliminated as possible causes. The presence of the *Micrococcus* was disregarded. In the light of present-day knowledge, it is apparent that this was, in all probability, a typical outbreak of micrococcic food poisoning.

Meyer (1934) also reported that, although micrococci were found in food which was epidemiologically implicated on the west coast prior to 1930, the laboratory merely reported inconclusive findings or failed to grasp the importance of the organisms when isolated.

Subsequent to 1930, conclusive evidence that micrococci may produce a filterable poison in foods has been presented by Dack, Cary, Woolpert, and Wiggers (1930). A yellow hemolytic *Micrococcus* was isolated by these investigators from a Christmas sponge cake which had caused illness in 11 individuals. Some of the same cake fed to a human volunteer caused symptoms of gastroenteritis. Sterile filtrates from this organism caused human volunteers to exhibit the same symptoms as were experienced by the victims of the outbreak.

Following this report, Jordan (1931), Jordan and Burrows (1933), and Jordan and Burrows (1934a,b) reported similar observations. Many filtrates from enterotoxigenic strains were tried on human volunteers and typical symptoms were produced.

These investigations finally established the health significance in food products of the presence of micrococci. Since the establishment of these organisms as causative agents of food poisoning, numerous reports of outbreaks have been published and there is little doubt as to the enterotoxicity of micrococci.

FOOD PRODUCTS INVOLVED

When the field once became established, a great variety of foods was incriminated in outbreaks of micrococcic food poisoning. Among these are various kinds of pie and cake, usually custard filled, ice cream, butter, cheese, éclairs, cream puffs, beef, chicken, chicken gravy, chicken and ham paste, chicken salad, frozen-pack vegetables, ground meat sandwiches, ham, headcheese sandwiches, kippers, milk, oysters, pastry, potato salad, sweet potato, candy, and tongue. It is apparent that almost any article of food which is not properly prepared and refrigerated may cause food poisoning of this type.

ENTEROTOXIGENICITY OF STRAINS

It had been noted by Barber that milk inoculated with a yellow *Micrococcus* which he obtained from the same milk which yielded white

toxigenic strains, was innocuous even after incubating at room temperature. When Dack, Cary, Woolpert, and Wiggers (1930) confirmed the fact that micrococci were toxigenic, they also were confronted with the thought that all micrococci might have this ability. They were, however, unable to induce ordinary stock strains of *Staphylococcus aureus* and *Staphylococcus albus* to elaborate the toxin. The fact that the enterotoxicity was confined to certain strains, or at least demonstrable by the methods used only in certain strains, was soon confirmed. Among 200 strains of micrococci capable of producing a potent exotoxin, Dolman (1934) found not a single enterotoxic strain. This led him to the conclusion that few strains were capable of producing the enterotoxin and that even these required such unusual environmental conditions to do so that micrococcic food poisoning would rarely be found. Jordan and Burrows (1934a,b) found that many different varieties of micrococci could produce the enterotoxin but Jordan (1930a,b) showed that they were of diverse origin, and Stritar and Jordan (1935) pointed out that they embraced few specific or varietal characters in common.

The large number of outbreaks of micrococcic gastroenteritis recorded in the literature is ample refutation of Dolman's original contention that it must be a rarity.

The failure on the part of Denys (1894) and Owen (1907) to establish, by means of human experimentation, a relationship between the micrococci they isolated and the symptoms they observed in victims of the outbreaks, in all probability accounts for the indifference to their findings. Although he did establish this relationship, the work of Barber (1914) did not become generally known until it was confirmed and augmented by Dack, Cary, Woolpert, and Wiggers (1930). Confirmation of the results obtained by Barber and by Dack and his associates was accomplished, using human volunteers.

Partly because the severity of the symptoms exhibited were so alarming and partly because susceptible laboratory animals were found, the use of human volunteers has been largely discontinued. Since no laboratory animal seems to be as susceptible as man when fed potent filtrates or cultures, the latter is still resorted to whenever inconclusive results are obtained by other means.

Difficulties are involved in the use of human volunteers because, as Jordan (1930a,b), Meyer (1934), Shaughnessy and Grubb (1937), and Minett (1938) have shown, individuals vary in their susceptibility to *Micrococcus* enterotoxin, some being entirely unaffected, as demonstrated by Grubb (1938). Still another difficulty is that some individuals develop an immunity as pointed out by Barber (1914) and Shaughnessy and Grubb (1937).

Among the first laboratory animals to be tried were the rodents. Generally unsuccessful results were obtained by Owen (1907), Dack, Cary, Woolpert, and Wiggers (1930), Jordan and McBroom (1931), and Corpening and Foxhall (1935) when potent filtrates or incriminated food was administered orally. Borthwick (1933), although he could obtain symptoms of poisoning only occasionally when he introduced the toxin directly into the stomach of guinea pigs and rabbits, obtained uniformly positive

results by this method if he first adjusted the reaction of their stomachs to pH 7.3. Meyer (1934), however, found this method not always effective and Dolman, Wilson, and Cockcroft (1936) were entirely unsuccessful in their efforts to use similar procedures. Intraperitoneal injection of cultures proved unsuccessful in the hand of Denys (1894), but Owen (1907) reported that administration in this manner led to death in white rats and guinea pigs while rabbits only became ill. Similarly, Meyer (1934) caused death in mice by intraperitoneal injection of sterile cultures. In view of the lethal effect upon many laboratory animals of untreated *Micrococcus* filtrates, these results may have been due to the lethal toxin and not to the enterotoxin. This may also explain the symptoms observed by Dack, Cary, Woolpert, and Wiggers (1930) and Dack, Jordan, and Woolpert (1931) following the intravenous inoculation of rabbits with potent filtrates. Dack *et al.* (1930), however, showed that even filtrates which had been heated at 100°C.(212°F.) for 30 minutes caused diarrhea and death in rabbits. Such heated filtrates would be devoid of lethal toxin. That the symptoms provoked in such cases are not due to the enterotoxin has been indicated by Roy (personal communication) who finds in *Micrococcus* filtrates a heat-stable lethal toxin which, however, is not identical with enterotoxin since it is not neutralized by antisera against enterotoxin. Singer and Hagan (1941) also found evidences of a lethal factor in heated filtrates. They attributed this residual toxicity to a greater resistance to heat of the factors of exotoxin than other investigators have found. Even prolonged boiling did not completely remove lethal toxin from their filtrates.

The effect on monkeys of *Micrococcus* cultures and filtrates as well as incriminated foods has also been studied. Barber (1914) was the first to attempt the use of monkeys. By feeding monkeys with milk cultures of the white *Micrococcus* he isolated, he was unable to provoke symptoms of poisoning. The next attempt to use monkeys was made by Dack, Cary, Woolpert, and Wiggers (1930). They were unsuccessful in inducing any symptoms in monkeys by feeding the components of the cake which provoked severe symptoms in human volunteers. Jordan and McBroom (1931) made the first experiments using sterile filtrates on monkeys. Their filtrates were administered by means of the stomach tube. Rhesus monkeys, they found, failed repeatedly to react to large doses of filtrates of proved toxicity. By using smaller monkeys of different species, they were able, by this same method, to provoke definite symptoms of poisoning in some.

About this time Dack, Jordan, and Woolpert (1931) showed that intravenous inoculation of filtrates would produce reactions in monkeys. One year later, Tanner and Ramsey (1932), failing to affect monkeys, concluded that they might not be suitable for studies of some phases of food poisoning. More success attended the investigations of Woolpert and Dack (1933) who, however, used a modified Burnet technique (a semisolid medium incubated in the presence of CO_2) for preparing the toxin and thus had more powerful filtrates. Such filtrates, when fed to rhesus monkeys, consistently caused similar symptoms to those seen in men. They concluded that, although monkeys, by oral administration, were not sensitive enough to the enterotoxin to be of value in detecting the amounts

of the enterotoxic agent usually occurring in foods, they were suitable for detecting this substance when it was present in large concentrations. Although they probably used this same method for preparing and administering their filtrates, Jordan and Burrows (1933) found that some monkeys did not react to the active principle. Meyer (1934) obtained good results. In 1935, these same authors confirmed the findings of Woolpert and Dack (1933) that monkeys are far less susceptible than humans to the action of the enterotoxin. Shaughnessy and Grubb (1936a,b) were the first investigators outside of the Chicago group to report favorably upon the method offered by Woolpert and Dack (1933). At the same time they confirmed the findings of Dack, Jordan, and Woolpert (1931) that monkeys would react to intravenous administration of potent filtrates. They also found that many monkeys were insusceptible to the enterotoxin. Davison, Dack, and Cary (1938) reported that intravenous injections of monkeys and kittens offered certain advantages over other methods of administering enterotoxin to these animals. The advantages found by them were an increase in the delicacy of the test, susceptible animals could be used repeatedly, and only small amounts of the test materials were required.

After the publication in 1936 of the Dolman kitten inoculation test for identifying enterotoxin, the disadvantages and imperfections of the use of monkeys assumed greater importance in the eyes of some investigators. Thus Shaughnessy and Grubb (1937) found monkeys to be unsatisfactory because they varied from day to day in susceptibility. They determined, by the use of apomorphine, that "the mechanism of vomiting in monkeys is not as sensitive as that of other mammals possessing a vomiting center." Minett (1938), however, although he found considerable variation, regarded monkeys as the best substitute for human volunteers. While Davison and Dack (1939) recognized the difficulties involved in the use of monkeys, they nevertheless recommended their use because they were able to obtain with them more positive results than by means of the kitten inoculation procedure of Dolman and because monkeys yielded accurate results by the feeding method. Chinn's faith in the use of monkeys was taxed when he observed vomiting in monkeys after they had been fed sterile nutrient broth [personal communication to Grubb reported in an article by Grubb (1938)].

As has been implied above, the only other laboratory animal which has been found useful in detecting enterotoxin in filtrates is the kitten. Before the method of Dolman, Wilson, and Cockcroft (1936) was published, various attempts, successful in the main, had been made to use kittens. The failures reported by some investigators were due mostly to the method of administration. Feeding portions of the suspected or incriminated food, as Owen (1907) and Corpening and Foxhall (1935) did, proved invariably unsuccessful. Milk cultures of the incriminated organism, as shown by Barber (1914), Ramsey and Tracy (1931), Tanner and Ramsey (1932), Meyer (1934), and Stone (1935), or of the sterile boiled filtrate, according to Dolman (1936), when administered orally were successful to some extent in producing some symptoms. Typical gastrointestinal symptoms

were noted by Owen (1907) after the intraperitoneal injection of cultures, and by Woolpert and Dack (1933) upon administration, in this manner, of sterile cultures of micrococci. These latter authors concluded that cats were too sensitive because they reacted with typical symptoms to a filtrate which consistently failed to affect humans or monkeys. On the other hand, Jordan and Burrows (1933) observed symptoms in a monkey inoculated with an acid ether extract of a potent filtrate, while cats and dogs were unaffected by intraperitoneal injection. In 1936 Dolman, Wilson, and Cockeroft published their method for detecting a potent filtrate. This method consisted of injecting kittens intraperitoneally with a filtrate from which the alpha and beta toxins had been removed by formalinizing or by boiling for half an hour. Although it had been shown by other investigators that monkeys were sometimes naturally refractory, these investigators had not encountered a naturally insusceptible kitten. This method was tried and quickly confirmed by Kupchik (1937), Geiger (1937), Gwatkin (1937), and Minett (1938). In 1938 Dolman and Wilson reiterated their claims, adding the observation that a filtrate from which the alpha and beta toxins had been removed by previous absorption with serum containing antitoxins for alpha and beta toxins would also produce symptoms in kittens. This method, usually using the heat-treated filtrate, has come to be accepted by most investigators as the easiest and most reliable method for detecting the presence of the enterotoxic substance. Objections have been raised to the use of kittens, however. Rigdon (1938) found that kittens sometimes vomited following the inoculation of the control medium, while they often failed to react to the injection of a potent filtrate. However, this latter statement must be considered carefully since he subjected the filtrates to 100°C. for two hours before inoculating kittens. Although it is accepted that the enterotoxin will withstand boiling for half an hour, the effect of a longer exposure has been shown to be destructive, according to Davison and Dack (1939). Stone (1937) also observed that kittens vomited when inoculated with sterile nutrient broth. Gwatkin (1937) noticed that one kitten which he inoculated with uninoculated boiled medium manifested some pain and uneasiness for about five minutes, but no lassitude or vomiting. The reliability of the kitten inoculation test is questioned further by Singer and Hagan (1941). They found that filtrates of the uninoculated medium caused vomiting in about 25 per cent of the tests, while filtrates of cultures presumed by them to contain enterotoxin, failed in nearly half the cases to cause vomiting. By repeated tests they found that some kittens proved resistant to all materials, while others vomited repeatedly with filtrates of broth cultures and uninoculated sterile medium. Perhaps because of observations similar to these, and because they have, in comparative tests, obtained more positive results with monkeys, Davison and Dack (1939) still feel that the kitten is inferior to the monkey for purposes of detecting enterotoxin. A review of the literature on the relative advantages and disadvantages of kittens and monkeys for detecting enterotoxin indicates that there is little choice between the two when reliability alone is considered. Considerations of ease in handling, maintenance, etc., make the kitten the animal of choice in most laboratories.

The technique of the kitten inoculation procedure has recently been elaborated upon by Dolman and Wilson (1938a, b, 1940). They also offer recommendations concerning the care and handling of kittens.

Attempts have been made to obtain reliable results by administering filtrates orally to kittens by means of a pipette. Minett (1938) and Jones and Lochhead (1939) report conflicting results by this method. Minett was unable to provoke symptoms by this method of administration and recommended the Dolman technique. Lochhead and Jones obtained good results and recommended the method. Since confirmation has not been forthcoming for either investigator's observations, it is impossible to comment upon the reliability of the method.

A warning has been sounded by Gwatkin (1937) that roundworm infestation, which is common in cats, may cause erroneous results since vomiting is one of the symptoms of the condition. This must be taken into account regardless of the method used to induce vomiting in kittens. This same author was troubled to know just which symptoms to connect with the effect of the enterotoxin. All the filtrates prepared by him produced a condition of lassitude in kittens and he wondered if some of the slight gastric disturbances were a part of this reaction.

A modification of the Dolman technique has been offered by Phatak and Pentler (1940). They find that this test can be rendered more objective and convenient by anesthetizing the kittens with sodium phenobarbital. A light anesthesia, lasting several hours, is produced during which the kittens are inoculated intraperitoneally with the heated filtrates. The kittens are then laid out in a row and observed for vomiting.

A few attempts to use dogs and puppies have been made. The first to attempt their use was Barber (1914) who found puppies refractory to oral administration of milk cultures of the implicated *Micrococcus*. Jordan and Burrows (1933) were also unsuccessful in provoking symptoms in dogs. They administered a potent filtrate intravenously. Five years later, Minett (1938) concluded that feeding tests on dogs, because of their variable susceptibility by the oral route were unsatisfactory. Rigdon (1938) observed that dogs vomited when injected with a sterile uninoculated control.

All of the above cited data indicate that there are only three animals which can be used for investigating micrococcic food poisoning. These three are man, monkeys, and kittens.

It seems to be agreed generally that human volunteers are the most reliable experimental subjects, as Dack (personal communication, 1935), Jordan and Burrows (1935), Dack and Cary (1937), and Grubb (1938) concluded that humans are more susceptible than any laboratory animal. The risk to life, however, is a deterrent. Jordan (1931) and Meyer (1934) expressed their fears concerning this problem. Their fears have been realized in reports of death attributable to *Micrococcus*-infected foods by Denys (1894), Vaughan and Novy (1902), Blackmann (1935) Fanning (1935), and Cogswell, Kilbourne, and Kuhns (1938). Another serious difficulty is that many human subjects are not susceptible to the action of the enterotoxin as indicated by Jordan (1930a,b, 1931), Meyer (1934), Shaughnessy and Grubb (1937), and Dack and Cary (1937). This, however, is a difficulty encountered also with monkeys and kittens. Most

laboratories do not have the facilities for taking care of human volunteers, nor can they obtain a constant volunteer supply.

By means of the technique developed by Woolpert and Dack (1933), monkeys have been found to give reliable results. Until the Dolman kitten inoculation test was devised, these animals were used successfully in all phases of the investigations. The difficulty again was that many laboratories were not equipped to maintain a colony of monkeys. Even for ones that were, the expense was great and the problem of making use of insusceptible animals was difficult. The only advantage of the monkey over the human volunteer is that a constant supply is always available.

The advent of the Dolman technique opened the way for many laboratories to conduct animal experimentation on this problem. The method has been adopted by many investigators, many of them finding it satisfactory. The wide acceptance of the method is due in part to the fact that any laboratory can maintain a few kittens. Neither special equipment nor trained helpers are needed to maintain them. The supply of kittens is variable, however, depending upon the season of the year. Since cats do not propagate under laboratory conditions, the supply must be obtained from outside sources. This introduces the possibility of disease in the colony and Wilson (1940) finds this to be one of the chief difficulties in maintaining a supply of kittens.

In the spring, summer, and fall when outbreaks of micrococcic food poisoning are most likely to occur, it is not difficult to obtain suitable kittens. During the winter, however, experimentation must cease unless human volunteers or monkeys are available.

Of the suitable laboratory animals the kitten commonly, therefore, will be found the most convenient to use. If due consideration is given to its imperfections and data obtained by its use is carefully interpreted, the kitten can probably be considered as reliable for detecting enterotoxin as any other method in use at the present time.

IN VITRO PROCEDURES FOR IDENTIFICATION OF ENTEROTOXINS

Simultaneously with the search for a susceptible laboratory animal many investigators sought an *in vitro* test for identifying micrococci of the food-poisoning type. These investigations, along cultural or serological lines, have been uniformly unsuccessful.

Stone (1935) introduced a method which he claimed would distinguish between enterotoxigenic micrococci and others. The method consisted of inoculating pure cultures of the suspected *Micrococcus* into specially prepared gelatin stabs, and incubating at 37°C.(98.6°F.) for exactly 24 hours. The tubes were then placed in a water bath at 21°C.(69.8°F.) until the control tubes hardened and were then observed for evidences of liquefaction. According to this author, any degree of liquefaction indicated a food-poisoning strain, while failure to liquefy was proof that the strain was non-enterotoxic. Later, Stone[2] introduced a modification of the method. The assumption by Stone in connection with the use of these methods could not, however, be substantiated by most other investigators. Chapman, Lieb, and Curcio (1937) have devised a series of media to be used in conjunction

[2] Pamphlet published by Digestive Ferments Co., Detroit, Michigan.

with the Stone reaction and other tests for identifying food-poisoning micrococci.

SOURCE OF FOOD-POISONING MICROCOCCI

The origin of these enterotoxigenic micrococci is still uncertain, but the evidence as found in the literature points strongly to bovine and human sources. This was recognized by Jordan and Burrows as early as 1934, who felt that raw milk or cream and human contact were the most likely sources, although they admitted that the ubiquitous distribution of micrococci made possible their entrance into food from a variety of sources.

The first indication of the bovine origin of enterotoxic micrococci was given by Denys (1894), as mentioned above, who implicated meat from a cow that had died of puerperal fever in an outbreak. Barber (1914) isolated food-poisoning micrococci from the milk taken directly, with all due precautions, from the udder of a cow. Thus, he also demonstrated the bovine origin of the organisms. Since Barber's report, numerous observations have been made confirming the fact that one of the most important sources of enterotoxigenic micrococci is the bovine udder. In many of these cases the possibility that the enterotoxigenic *Micrococcus* gained entrance to the milk from some other source than the udder of the cow is apparent. Ramsey and Tracy (1931) and Tanner and Ramsey (1932) reported the isolation of such an organism from raw milk delivered to a commercial milk plant. Crabtree and Litterer (1934) and Dolman, Wilson, and Cockcroft (1936) likewise isolated such organisms from raw milk. Shaughnessy and Grubb (1936a,b) had similar experiences, but they found (1937) that outbreaks usually occurred where one or more of the cows which produced the milk had micrococcic mastitis. That the source of these organisms was in all probability the cow's udder was shown by Gwatkin (1937) who isolated them from normal udders as well as from the udders of cows in which mastitis was caused by micrococci. This was corroborated by Minett (1938) who found a large proportion of micrococci from normal udder milk as well as from cases of acute and chronic mastitis to be enterotoxic.

From these findings it seems that the bovine source of enterotoxin-producing micrococci has been established beyond any reasonable doubt.

Minett (1938) showed that the enterotoxin subsisted in cheese made with infected raw milk, and Shaughnessy and Grubb (1937) found ice cream made with raw milk from cows with micrococcic mastitis to be enterotoxigenic. Therefore, not only fluid milk and cream but milk and cream to be used for the manufacture of other foods should be properly pasteurized in order to avoid food poisoning.

The other important source of enterotoxigenic micrococci is man. The importance of this source of contamination was first indicated by Jordan (1903a,b). He isolated enterotoxigenic strains from the normal human throat and from a case of septicemia in man. Several cases have been reported in which the persons who prepared the incriminated food or who worked around it were found to have boils or infections on their fingers, hands, forearms, or bodies. In none of these cases, however, was an attempt made to show that the organisms isolated from the food and the organism from the pustules were similar.

Recognizing the importance of the human source, McBurney (1933), although he was unable to trace the source of the organisms involved in an outbreak he investigated, postulated that coughing and sneezing by bakery personnel while making and handling products, might easily be a source of contamination. Such a possibility had been indicated by Jordan (1930a,b) who found enterotoxic strains of micrococci in the normal human throat, and by Chapman, Berens, and Stiles (1941) who obtained "culturally typical" food-poisoning micrococci from nose and throat cultures of persons suffering from low-grade infections. Experimental evidence of such a possibility was advanced by Bransfield (1937). He isolated culturally and morphologically identical strains of micrococci from the incriminated food and from the nose and throat of one of the workers in the bakery. The correlation was particularly definite because non-lactose-fermenting strains were involved. The failure to ferment lactose is unusual among both enterotoxic and human pathogenic strains, and hence a common origin of the strains was almost certain. Similar observations were reported by McCastline, Thompson, and Isaacs (1937).

Until it was shown by animal experimentation that strains from two such sources were toxigenically as well as culturally and morphologically identical, the theory of the human source in such cases was subject to criticism. This was accomplished by Roberts, Deadman, Elliott, and Wilson (1938) and by Roberts (1939). The individuals to whom the strains involved were traced gave no visible evidence of the infection. Furthermore, the infection persisted in spite of medical treatment in one individual who had conveyed the infection to others working with the food. He was designated a carrier after a second outbreak had been traced to the same bakery.

It was pointed out by Haynes (1935) that humans normally harbor enterotoxigenic strains of micrococci under their finger nails and that food might become contaminated from such sources. It thus becomes apparent that healthy individuals might be the source of an outbreak.

STIMULATION AND REACTIVATION OF ENTEROTOXICITY

The ability of micrococci to produce the enterotoxin varies from time to time. This was first reported by Jordan (1931). Various other investigators were puzzled by this phenomenon and Dolman (1934) concluded that only a few strains were capable of elaborating the toxin and that their potency was only temporarily increased to a point where it could be detected by certain special artificial conditions. Other investigators found that the environmental conditions in many foods were of such a nature that the toxin could be produced in them naturally. However, it was recognized that the enterotoxin-producing ability of strains was often lost or greatly lessened by growth on artificial media, as indicated by Dack (1937) and Grubb (1938). It was reported by Jordan and Burrows (1935) that growth under CO_2 reactivated strains which had lost their potency while certain strains which had never before yielded toxic filtrates were caused to do so under these conditions. By successive transfers on media containing starch the property of producing enterotoxin was restored to strains which could not be reactivated by growth under CO_2. Furthermore, several

other strains which had never before yielded enterotoxic filtrates were found to do so after being subjected to this procedure. Stone (1936, 1937) reported a higher percentage of reactivation by use of a medium which combined his gelatin-agar with Chapman's crystal violet principle than with the starch medium of Jordan and Burrows. He later (personal communication) found that his observations could not be repeated and concluded that there was no certain way of reactivating strains which had lost their enterotoxigenic capacity.

STERILE *MICROCOCCUS* FILTRATES

It has been established by various investigators that micrococci are capable of elaborating a variety of substances which are thought to be associated with their pathogenicity. These substances, most of which may be classed as toxins, are designated by names which indicate their actions upon the host or the tissues and fluids of the host. Thus the hemotoxins (or hemolysins) lyse or dissolve red blood corpuscles, dermotoxin (dermonecrotoxin) causes necrosis of the skin, lethal toxin causes death to certain animals when intravenously injected, enterotoxin irritates the enteron or digestive tract, leucocidin destroys leucocytes, coagulase causes clotting of blood plasma, and fibrinolysin liquefies fibrin. The first three of these factors (hemotoxin, dermotoxin, and lethal toxin) usually are designated under one head as exotoxin. This is for the purpose of distinguishing them from enterotoxin which has also been called gamma toxin by Dolman and Wilson (1938a,b,c). Since enterotoxin may be considered as an exotoxin, this terminology may lead to confusion. In the absence of more accurate terms to differentiate between these factors, however, we shall adopt the above nomenclature.

There is evidence that other factors are present in sterile *Micrococcus* filtrates which also possess the characteristics of true exotoxins. Little is known about them, however, and their importance is questionable. The observations made by Roy (personal communication) in this regard are especially interesting because of the light they shed on some of the early work with enterotoxigenic filtrates. He has found evidence of a heat-stable factor which is lethal for rabbits but which is not exotoxin, inasmuch as it is not neutralized by antitoxin against exotoxin. Singer and Hagan (1941) found evidence of lethal toxin in their filtrates, even after prolonged boiling, but they considered it to be residual toxicity owing to exotoxin. With these reports in mind it is interesting to note that Dack, Cary, Woolpert, and Wiggers (1930) reported death occurring in rabbits following the intravenous injection of filtrates which had been heated for 30 minutes at 100°C. This they considered to be caused by the enterotoxin which they had previously demonstrated in these filtrates by feeding them to human volunteers. The heat-stable lethal factor found by Roy, however, is not enterotoxin since antitoxin against enterotoxin does not neutralize it.

RELATIONSHIP OF ENTEROTOXIN TO OTHER TOXIC FACTORS PRESENT IN *MICROCOCCUS* FILTRATES

Enterotoxin is distinct from the exotoxic factors with which it is associated in *Micrococcus* filtrates. This was proven by Woolpert and Dack

(1933) who pointed out the differences in heat resistance and absorptive capacity between enterotoxin and exotoxin. More conclusive proof of this difference was furnished upon failure to neutralize the enterotoxin present in a filtrate containing exotoxin with an antitoxin against exotoxin. While they were thus able to distinguish between enterotoxin and the components of exotoxin (hemotoxin, dermotoxin, and lethal toxin), they were unable to prepare filtrates containing enterotoxin free of the factors of exotoxin. However, enterotoxigenic filtrates have been prepared which contained no hemotoxin, by Woolpert and Dack (1933), Stritar and Jordan (1935), and Dennison (1936). On the other hand, Dolman (1934) prepared many exotoxic filtrates free from enterotoxin. A rough correlation was found by Woolpert and Dack (1933) between the quantity of enterotoxin in a filtrate and the amounts of the other factors which were also present.

Since the only criterion for determining the presence of enterotoxin in a filtrate is animal experimentation, it is important to know that symptoms might be provoked in animals by exotoxin. Dolman (1934), by feeding highly potent exotoxins to human volunteers, has shown that they provoke no symptoms when administered orally. Therefore, feeding experiments are not complicated by the presence of exotoxin. The usual procedure is parenteral injection of laboratory animals, however, and under these circumstances exotoxin may cause symptoms similar to those provoked by enterotoxin, according to Davison, Dack, and Cary (1938) and Blair (1939).

Complications arising from the presence of exotoxin in filtrates is avoided by destruction of the exotoxin with heat or formalinization or by neutralization with antitoxin. In the past, however, the possibility of such complications was not recognized and one cannot help but wonder if some of the results which led to the controversy over the resistance to heat of enterotoxin might not have been due to just such a complication. For instance, Jordan (1930a,b) reported that enterotoxin was destroyed in his filtrates by boiling and either destroyed or greatly weakened by exposure to 60-65°C.(140-149°F.) for 30 minutes. Since he used human volunteers, however, the heat lability of his enterotoxin may have been due to some other factor. Whatever the cause, it was apparently discovered by Jordan for he later confirmed the work which showed enterotoxin to be heat stable as reported by Jordan, Dack, and Woolpert (1931).

ENTEROTOXIN AND EXOTOXIN SEPARATION

Since *Micrococcus* filtrates usually contain a variety of toxic factors, it is often difficult to determine which factor has caused a given reaction. The similarity of symptoms provoked in experimental animals by exotoxin and enterotoxin has been mentioned. Singer and Hagan (1941) have shown that even prolonged boiling does not completely destroy the lethal toxin and Roy (personal communication) has found evidences of a heat-stable lethal factor in some filtrates. Therefore, some doubt still may exist if the enterotoxin is the cause of symptoms observed in experimental animals even though the filtrate is boiled before administration. If enterotoxin

could be separated from the other factors of a filtrate, its properties could more certainly be determined and an insight into its chemical nature might result.

Such a separation was accomplished by Jordan and Burrows (1933). By extraction of an aqueous solution containing enterotoxin with ethyl ether or chloroform they obtained an extract which, after all traces of the solvent had been removed, provoked typical symptoms of poisoning when fed or intravenously injected into monkeys. Davison and Dack (1939), while they did not question the extraction with ethyl ether, denied the solubility of enterotoxin in chloroform. They assayed the enterotoxicity of their fractions by intracardial injection of kittens and by monkey feeding. The supernatant fluid resulting from their extraction was consistently positive, but the chloroform layer was always negative. They attribute the results obtained by Jordan and Burrows (1933) to the fact that these latter workers administered the residue of an *emulsion* of chloroform and filtrate, and thus were not sure whether the enterotoxin was really dissolved or only adsorbed on the chloroform globules. Davison and Dack (1939) destroyed the emulsion by centrifugalizing and thus prevented any difficulties arising from adsorption. Their implication that the enterotoxin is insoluble in chloroform is, however, not justified. Although this may be true, and it is improbable in view of the solubility of enterotoxin in ethyl ether, their methods cannot properly lead to such a conclusion. The basis of the process of extraction is the Distribution Law which states that, in a system of two liquid layers made up of two immiscible or slightly miscible components a third substance, soluble in both layers, distributes itself between the two layers in a constant ratio (the distribution coefficient) which is independent of the total amount of dissolved substance present. If the distribution coefficient is high, and the enterotoxin is more soluble in water than in chloroform, the quantity of enterotoxin extracted by the chloroform would be too small to detect by the crude methods available. On the other hand, a pure anhydrous enterotoxin might be dissolved easily in chloroform. The residue of the ethyl ether extract which remains after the solvent has been driven off, although probably far from pure, might more properly be used in determining the solubility of enterotoxin.

Although they controlled pH, time, temperature, etc., Davison and Dack (1939) obtained variable results in their attempts to dissolve the enterotoxin in ethyl alcohol. Unlike the exotoxin, enterotoxin survived the alcohol treatment, but it appeared sometimes in the filtrate and sometimes in the precipitate. They concluded that some other factor than those they controlled must control alcoholic precipitation.

The solubility of enterotoxin in strong salt solution was also investigated by Davison and Dack (1939). By feeding fractions obtained in this way to monkeys they determined that the enterotoxin is consistently "salted out" from saturated solutions of ammonium sulfate but not from those half saturated.

Although enterotoxin may be obtained in more or less pure form by extraction with ethyl ether or by "salting out" with ammonium sulfate, no work other than that reported has been conducted. Perhaps now that

the presence of complicating, heat-stable factors has been discovered, these methods for purifying the enterotoxin will be utilized.

ACTION OF CHEMICAL AGENTS UPON ENTEROTOXIN

The action of chemical agents upon enterotoxin is a subject which has, for the most part, been neglected. Fragmentary reports in the literature seem to indicate that the enterotoxin is a conjugate protein of the lipoprotein type. Its lipoidal nature is indicated by its ether solubility and its instability towards dilute sodium hydroxide. Its insolubility in saturated solutions of ammonium sulfate, as shown by Davison and Dack (1939), and its susceptibility to trypsin, according to Minett (1938), are indications of its protein nature. Beyond this nothing is known except that the enterotoxicity of the enterotoxin is not altered by alcohol, as shown by Davison and Dack (1939); formaldehyde, according to Dolman, Wilson, and Cockcroft (1936) and Minett (1938); chlorine, according to Dack, Jordan, and Woolpert (1931); and acid (pH 5.0) or rennet, as shown by Minett (1938).

EFFECT OF PHYSICAL AGENTS UPON ENTEROTOXIN

The most prominent physical property of enterotoxin is its heat stability. Eleven years ago there was a brief controversy over this point, Jordan (1930a,b) contending that boiling destroyed enterotoxin and that 60-65°C. for half an hour either destroyed or greatly weakened it and Dack, Cary, Woolpert, and Wiggers (1930) holding that it resisted boiling for half an hour. Jordan, Dack, and Woolpert (1931) soon discovered the error and confirmed the resistance of enterotoxin to boiling. This was confirmed by Dolman, Wilson, and Cockcroft (1936), Minett (1938), and Dack (1939). Rigdon (1938) found that boiling for two hours destroyed the enterotoxin and Davison and Dack (1939), besides confirming the instability of the enterotoxin to prolonged heating, found that autoclaving decreased its potency. Jordan and Burrows (1933) found that it was unstable to heat in acid solutions (N/100 HCl).

Most exotoxins tend to deteriorate when they are stored, even when held at low temperatures. Enterotoxin, besides differing from ordinary exotoxin in its resistance to heating, retains its toxicity when stored at low temperatures for two months, as reported by Jordan, Dack, and Woolpert (1931) and Jones and Lochhead (1939).

Other physical properties which have been investigated are the ability of enterotoxin to dialyze, its adsorptive capacity, and its volatility. Although it diffuses freely into the culture medium, enterotoxin diffuses only slightly through collodion, according to Minett (1938) and Jordan and Burrows (1933). It is partially adsorbed on Seitz pads during filtration, but to a lesser extent than the other toxic factors present in *Micrococcus* filtrates, as shown by Woolpert and Dack (1933). Lastly, it was found by Jordan and Burrows (1933) that enterotoxin would not distill.

ANTIGENICITY OF ENTEROTOXIN

Woolpert and Dack (1933) were the first investigators to demonstrate the antigenicity of enterotoxin. They were able to accomplish active im-

munization of rhesus monkeys without difficulty, but all their attempts to immunize them passively failed. Although Dolman, Wilson, and Cockcroft (1936) reported that preliminary experiments indicated that enterotoxin was antigenic, adequate confirmation of this fact was not forthcoming until Dolman and Wilson (1938a, b) and Minett (1938) published their observations. Dolman and Wilson were further able to demonstrate a flocculation reaction involving the enterotoxin and the specific antibody. Davison and Dack (1939) also have shown that kittens experience no symptoms when injected intraperitoneally with a mixture of enterotoxin and a specific antiserum. When, however, such a mixture is injected directly into the blood stream of a monkey or a kitten, typical food-poisoning symptoms occur. These investigators concluded that the presence of whole blood, extravascularly or intravascularly, prevented the neutralization of the enterotoxin by antiserum prepared against it.

Dack (1937) found that oral administration of potent filtrates to man and rabbit was ineffective in stimulating the production in them of antibodies against the enterotoxin. Barber (1914) reported, however, that individuals who had repeatedly drunk the milk he incriminated in an outbreak seemed to have developed some tolerance to the toxin. Similarly Dack, Jordan, and Woolpert (1931) found that human volunteers fed gradually increasing doses of potent filtrates appeared to develop some tolerance to the poison. Apparently some degree of immunity may be developed in individuals by oral administration of the enterotoxin, but consistent results are only obtained by parenteral inoculations.

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EFFECTS OF METHOD OF BLANCHING AND TEMPERATURE OF STORAGE ON NUTRITIVE VALUE OF DEHYDRATED CABBAGE[1]

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Cabbage has been one of our most important dehydrated vegetables for use by the Armed Forces and Lend-Lease. The ascorbic acid content of cabbage is very high and it has been found to remain high even after dehydration.

Considerable disagreement exists as to the best method of blanching cabbage for dehydration. Much work has been done to show that cabbage should be dehydrated without blanching. Beckley and Notley (1941) and Sugihara and Cruess (1942) recommended that cabbage should be dehydrated without blanching. The latter workers found a higher yield of the dehydrated product when the vegetable was not blanched. Tressler, Moyer, and Wheeler (1943) dehydrated unblanched cabbage for four hours and found a loss of only 20 per cent of the ascorbic acid during dehydration. The fresh cabbage contained 4.40 mg. of ascorbic acid per gram, dry basis, while after dehydration there was 3.56 mg. per gram.

Steam blanching has been widely recommended in the United States in preference to water blanching. Hollingshead (1943) found that steam-blanched vegetables lost less ascorbic acid than the same vegetable which had been water blanched. Cabbage lost 14 per cent of this vitamin when steam blanched and 51 per cent when water blanched.

A recent improvement in blanching cabbage has been made possible by the use of a sulfite spray or dip. Cruess, Balog, Friar, and Lew (1944) found that dehydrated cabbage which had been treated with sulfite had a better color and flavor and could be dried at a temperature 20°F. or more above that used for cabbage which had not been sulfited. Sulfiting also prolonged the storage life of the dehydrated products.

Allen, Barker, and Mapson (1943) made an extensive study of the effect of different types of blanching on 110 lots of 14 varieties of cabbage. They found that unblanched cabbage gave a very poor product after dehydration. Steam blanching had no advantage and resulted in a poor product. Water blanching gave a product which was less satisfactory than that which had been sulfited prior to water blanching. In this latter method, dehydrated cabbage was prepared without noticeable loss of color, flavor, or ascorbic acid content. The cabbage was shredded and blanched by scalding for two or three minutes in a volume of 0.22 per cent Na_2SO_3 solution. The total loss of ascorbic acid owing to blanching

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and dehydration was 70 per cent if the cabbage was water blanched, and 52 per cent if treated with sulfite before blanching.

The sulfite treatment during the blanching of cabbage has been found by Mackinney, Friar, and Balog (1943) to prevent definitely the development of hay flavors when the dehydrated product was held at 32.2°C. (90°F.). The retention of ascorbic acid and carotene was also much greater.

High storage temperatures have been found generally to cause higher losses of ascorbic acid from dehydrated cabbage. Tressler, Moyer, and Wheeler (1943) found ascorbic acid losses increased as storage temperature increased from —40 to 24°C.(—40 to 75°F.).

EXPERIMENTAL PROCEDURE

Statistical Design of Experiment:[2] A factorial design of experiment was used for the study. An amount of Marion Market variety of cabbage sufficient for the complete experiment was produced and harvested under the usual commercial growing conditions. The cabbage was divided into six lots to be used for that number of replications. Replicates 1 and 2 were dehydrated one day after harvest; Replicates 3 and 4 were held six days before dehydration; and Replicates 5 and 6, nine days. During this time the cabbage was stored at the usual household refrigerator temperature until used for the experiment. An amount of cabbage sufficient for one replicate was trimmed, cored, and cut radially in shreds one-fourth to one-half inch in thickness. It was mixed well and a total of 10 samples was taken. Two samples were blanched by each of five methods. After dehydration, one of these samples was stored by one method and the other by another method for six months. This procedure was repeated for each of the six replicates, making a total of 60 samples for the entire experiment.

All data were analyzed by the analysis of variance. If the value for F was significant at the 5.0, 1.0, or 0.5 per cent level, the difference among means necessary for significance was calculated at the 5.0 per cent level.

Blanching Methods: The five blanching methods used for the 1,100-gram samples of cabbage were (1) blanched in 3,000 ml. of boiling water (an amount just sufficient to cover the raw cabbage) for 12 minutes with the lid off; (2) dipped in a one per cent Na_2SO_3 solution for 30 seconds, drained, then blanched 12 minutes in 3,000 ml. of boiling water with the lid off; (3) blanched for 22 minutes in a rack suspended in steam at the temperature of boiling water; (4) dipped in a one per cent Na_2SO_3 solution for 30 seconds, drained, then blanched 22 minutes in steam; and (5) unblanched to be used as check lots. The time of blanching was measured from the time the cold vegetable was placed in the boiling water or steam. The length of time the cabbage was to be blanched had been determined by the benzidine test of Proctor (1942) as that time necessary for inactivation of peroxidase.

Dehydration Process: The 20 samples from the two replicates, dehydrated the same day, were randomized for location in the dehydrator. The dehydrator, Eheart and Sholes (1945a), was maintained at a temperature

[2] The statistical design of experiment was planned by Dr. Boyd Harshbarger, Statistician, Virginia Agricultural Experiment Station, and the analysis of the data was carried out under his supervision.

of 48.9 to 57.2°C.(120 to 135°F.). The cabbage was dried until brittle which required 13.5 hours for the first drying period and 11.3 hours for the second and third periods. The cabbage was ripened in an airtight tin can for 24 hours in order to bring about equal distribution of moisture within each sample.

Storage Methods: After ripening, portions of the dehydrated samples were stored six months by each of two methods: (1) in fruit jars in a refrigerator at an average temperature of 5.6°C.(42.1°F.) and an average relative humidity of 59 per cent; (2) in fruit jars at an average room temperature of 24.1°C.(75.4°F.) and an average relative humidity of 42.2 per cent. The jars had three-piece lids with glass tops and rubber rings which fit the top of the jar.

Analytical Methods Used: The dehydrated cabbage was analyzed for moisture, calcium, and phosphorus. Losses of ascorbic acid were studied after blanching, after dehydration, and after storage for six months.

For moisture determinations, the dehydrated cabbage was ground in a hammer mill and dried to constant weight in circulating air at an oven temperature of 72°C.(161.6°F.). Calcium and phosphorus were determined by the official methods of the Association of Official Agricultural Chemists (1940).

The Morell (1941) method was used for all ascorbic acid determinations. Difficulty was encountered in the sulfited samples since SO_2 slowly reduced the indophenol dye and thus caused drifting of the galvanometer. It was found that the addition of acetone, as suggested by Mapson (1941), was successful in preventing the interference of SO_2. An amount of acetone to give a 20-per cent solution was added to the filtrates when they were made to volume to be read in the photolometer.

TABLE 1

Effect of Method of Blanching Cabbage on Its Retention of Dry Matter During Blanching and on Its Calcium and Phosphorus Contents

Method of blanching [1]	Retention of dry matter	Calcium content	Phosphorus content
	pct.	*mg./gm.*	*mg./gm.*
Tap water	64.18	5.08	3.33
Na_2SO_3—tap water	64.12	4.92	3.23
Steam	95.61	4.10	3.31
Na_2SO_3—steam	94.10	3.93	3.27
Unblanched (raw)	.....	4.02	3.44
Differences necessary for significance at 5% level	3.72	0.32	..... [2]

[1] All values given are means of 12 samples. Figures were calculated on dry basis. [2] No significant difference among means.

RESULTS AND DISCUSSION

The effect of the method of blanching cabbage on its calcium and prosphorus content and its retention of dry matter during blanching is shown (Table 1). The calcium content of the raw cabbage was significantly increased by both methods of blanching in water, but there was no difference among samples of cabbage blanched in steam, those dipped in one per cent Na_2SO_3 solution prior to steam blanching, and raw cabbage. The

calcium content was increased from 4.02 mg. per gram [3] for the raw cabbage to 5.08 for the water blanched and 4.92 for that dipped in Na_2SO_3 solution before water blanching. The difference between the latter two values was insignificant. This increase in calcium during blanching, owing to absorption of this mineral from tap water, had been noted earlier for green beans, Eheart and Sholes (1945a), and for sweet potatoes, Eheart and Sholes (1945b). The tap water used contained 45 parts per million of calcium. The calcium content of steam-blanched cabbage, that dipped in Na_2SO_3 prior to steam blanching, and the unblanched were, respectively, 4.10, 3.93, and 4.02 mg. per gram. The phosphorus content of cabbage was not affected significantly by any method of blanching and ranged only from 3.23 to 3.44 mg. per gram for the five methods of blanching. The retention of solids during blanching was much greater for cabbage which was steam blanched than that which was water blanched. The Na_2SO_3 dip did not make a significant difference in retention of solids in either case. The steam-blanched cabbage retained 94.10 and 95.61 per cent of the original solids while the water-blanched cabbage retained only 64.12 and 64.18 per cent. This difference in retention of dry matter owing to method of blanching would result in a considerable difference in the yields of dehydrated cabbage blanched by the different methods. The average amount of the dehydrated product from 100 pounds of raw cabbage would be 5.28 pounds for the water blanched, 5.28 pounds for that dipped in Na_2SO_3 then water blanched, 7.85 pounds for the steam blanched, 7.74 pounds for that dipped in Na_2SO_3 then steam blanched, and 7.86 pounds for the unblanched.

The effect of method of blanching on the retention of ascorbic acid in dehydrated cabbage is shown (Table 2). When the cabbage was analyzed immediately after blanching it was found that all methods of blanching caused a significant loss of ascorbic acid from the raw samples. The raw cabbage contained 5.787 mg. per gram while the blanched cabbage ranged from 2.354 to 3.185 mg., depending on the method of blanching used. Water-blanched cabbage contained significantly more ascorbic acid (3.185 mg. per gram) than cabbage blanched in steam (2.354 mg. per gram). The Na_2SO_3 dip significantly increased the ascorbic acid content of steam-blanched cabbage (2.736 mg. per gram) but it was still significantly lower than that of water-blanched cabbage. The average content of ascorbic acid in the blanched cabbage was 2.821 mg. per gram which represented 48.75 per cent of the original ascorbic acid in the raw cabbage, a highly significant loss owing to blanching.

After dehydration (Table 2) the effect of method of blanching on the ascorbic acid content of cabbage was more definite. When the ascorbic acid content was reported as milligrams per gram, dry basis, the cabbage which had been dipped in a one-per cent solution of Na_2SO_3 and then water blanched was significantly the highest, containing 2.378 mg. That which had been water blanched was next, containing 1.488 mg. The cabbage blanched in steam, that dipped in a Na_2SO_3 solution and then blanched in steam, and the unblanched or raw cabbage were not sig-

[3] All data are reported on the dry basis unless otherwise stated.

nificantly different, ranging from 0.601 to 0.811 mg. per gram. Since the water-blanched cabbage lost so much more dry matter (Table 1) than the steam-blanched, the ascorbic acid content was calculated on the basis of the total dehydrated lot which came from 1,100 grams of raw cabbage. Results on this basis, however, were no different from those calculated on the per gram basis. The Na_2SO_3 plus water blanch gave the highest results; the steam blanch, Na_2SO_3 plus steam blanch, and no blanching gave the lowest results.

TABLE 2

Effect of Method of Blanching and Storing Dehydrated Samples on Ascorbic Acid Content of Cabbage After Blanching, Dehydration, and Storage (Dehydrated Samples) and on Moisture Content After Dehydration and Storage (Dehydrated Samples)

Factors studied	Ascorbic acid content after					Moisture content after	
	Blanching	Dehydration		Storage (dehydrated samples)		Dehydration	Storage (dehydrated samples)
				Refrigerator	Room		
Method of blanching[1]	*mg./gm.*	*mg./gm.*	*mg./lot*	*mg./gm.*	*mg./gm.*	*pct.*	*pct.*
Tap water	3.185	1.488	83.793	1.560	0.343	8.33	8.08
Na_2SO_3—tap water	3.008	2.378	132.746	2.602	0.338	8.53	8.47
Steam	2.354	0.707	58.685	1.117	0.168	8.36	8.05
Na_2SO_3—steam	2.736	0.811	66.110	1.167	0.015	8.46	8.43
Unblanched (raw)	5.787	0.601	52.317	1.035	0.228	8.51	8.42
Difference necessary for significance at 5% level	0.369	0.236	17.469	0.445	0.143	.....[3]	.....[3]
Method of storing[2]							
Fruit jars—refrigerator	.....	.....	.....	1.493		.....	8.33
Fruit jars—room	.....	.....	.....	0.219		.....	8.25
Difference necessary for significance at 5% level	.....	.....	.....	0.715		.....	.....[3]
Mean	2.821[4]	1.197	.....	.....		8.44	8.29

[1] All values are means of 12 samples. Figures for ascorbic acid are on the dry basis. [2] All values are means of 30 samples. [3] No significant difference among means. [4] Excluding unblanched samples.

The average content of ascorbic acid of dehydrated cabbage was 1.197 mg. per gram dry basis, which represented 20.68 per cent of the original content of the raw cabbage, a highly significant loss owing to dehydration.

When the amounts of ascorbic acid in the dehydrated samples were calculated back to 100 grams of the original raw cabbage (an average serving) it was found that the following amounts of ascorbic acid were present after dehydration: 7.9 mg. in the water blanched, 12.6 mg. in the Na_2SO_3 plus water blanched, 5.7 mg. in the steam blanched, 6.4 mg. in the Na_2SO_3 plus steam blanched, and 4.7 mg. in the unblanched.

The temperature at which the dehydrated samples were stored (Table 2) had a decided effect on the retention of ascorbic acid. The samples stored in the refrigerator were significantly higher in ascorbic acid content after six months of storage than they were before, while those stored at room temperature lost the major part of their ascorbic acid. The reason for the gain in ascorbic acid content at refrigerator temperature, 5.6°C.

(42.1°F.), is not understood at present. Further study on the dehydro form of the vitamin, and on the enzymes still present after blanching might reveal the cause for this increase. The different methods of blanching rank approximately the same in the refrigerator-stored samples as they did after dehydration, i.e., the Na_2SO_3 plus water-blanched samples were highest (2.602 mg. per gram); the water blanched, steam blanched, and Na_2SO_3 plus steam blanched were next (1.117 to 1.560 mg. per gram); and the unblanched samples were lowest (1.035 mg. per gram). The retention of ascorbic acid in stored, dehydrated cabbage which had been treated with the Na_2SO_3 plus water blanch was 45 per cent of the raw. The samples which were room stored showed no consistency in their ascorbic acid content. The water-blanched samples were somewhat higher (0.338 and 0.343 mg. per gram) than the steam blanched (0.168 mg.) and the Na_2SO_3 plus steam blanched were the lowest of all (0.015 mg. per gram). The reduction in ascorbic acid content was so great at room temperature that it should never be recommended for storage of cabbage.

Although different methods of blanching and two methods of storage were used, there were no significant differences in the moisture content of any of the dehydrated cabbage samples, either immediately after dehydration or after six months of storage. The average moisture content after dehydration was 8.44 per cent and after six months of storage, 8.29 per cent. The change of moisture content during storage was not significant.

From the standpoint of nutritive value, the data herein presented would indicate that the best method of blanching cabbage for dehydration is dipping in a one-percent solution of Na_2SO_3 for 30 seconds, then blanching in water until the peroxidase is destroyed. This method caused greatest retention of ascorbic acid, absorption of calcium from the blanching water, and no loss of phosphorus. The next best method would be water blanching and the poorest methods would be steam blanching, Na_2SO_3 dip plus steam blanching, and no blanching.

From the standpoint of palatability, the cabbage in the experiment which was treated with Na_2SO_3 and then water blanched was best since, after cooking, the dehydrated samples had a bright green color. The flavor was good although slightly bland. The steam-blanched samples were poorest because they were brown in color and had a distinct hay flavor.

From the standpoint of cost of production, the steam-blanched, dehydrated cabbage would be cheaper because 100 pounds of raw cabbage would produce 7.85 pounds of dehydrated cabbage if steamed blanched and only 5.28 pounds if water blanched.

SUMMARY AND CONCLUSIONS

Water-blanched cabbage, whether dipped in a one-per cent solution of Na_2SO_3 or not, absorbed calcium from the blanching water. Steam-blanched cabbage was no higher in calcium than the raw cabbage.

Neither water blanching nor steam blanching caused a significant loss of phosphorus from raw cabbage.

Water blanching preceded by a 30-second treatment in a one-per cent Na_2SO_3 solution is by far the best method of blanching cabbage for preservation of ascorbic acid. Cabbage blanched by this method contained

2.602 mg. per gram of ascorbic acid after dehydration and storage for six months in a refrigerator. Other samples contained as little as 1.035 mg. under similar conditions.

The temperature at which dehydrated cabbage was stored for six months was found to be very important in retention of ascorbic acid. Samples stored in the refrigerator actually gained a small amount of ascorbic acid while those stored at room temperature suffered large losses of this vitamin.

Water-blanched, dehydrated cabbage was much greener than steam-blanched and had a much better flavor.

Water-blanched cabbage would yield less after dehydration than steam-blanched because more dry matter was lost owing to solution during blanching.

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STORAGE AND MICROBIAL ACTION UPON VITAMINS OF THE B-COMPLEX IN PORK[1]

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Information regarding the changes which occur in foods during storage is fundamental to studies of nutritive value. Nevertheless until recently there have been few attempts to determine the effects of storage upon the nutrients, most attention being focused upon the retention of the original color, texture, and flavor and upon the prevention of bacterial spoilage. However, the fact that changes readily occur in the physical and organoleptic qualities of foods is indicative of enzyme activity. Since enzymes and vitamins are so frequently interrelated, it seems probable that changes in the concentrations of certain of the vitamins may occur as the food is stored.

Losses of ascorbic acid and carotene from fruits and vegetables have been demonstrated—Esselen, Lyons, and Fellers (1942); Gleim, Tressler, and Fenton (1944); Harris and Mosher (1941); Mack, Tapley, and King (1939); Mack, Tressler, and King (1936); Manville, McMinis, and Chuinard (1936); Mayfield and Richardson (1940); Tressler, Mack, and King (1936)—but less attention has been given to the vitamins in other foods, especially meats. In one limited investigation Schweigert, McIntire, and Elvehjem (1944) found that in fresh hams the niacin and thiamin content decreased by eight per cent and the riboflavin content decreased by 15 per cent during two weeks' storage at —4°C.(24.8°F.).

In the present investigation an attempt has been made to determine more exactly the effect of storage upon the vitamin-B complex in pork. Since only slight changes were expected, some of the samples were stored until partially putrefied, thus permitting measurement of the rates of change of vitamin content over relatively long periods and correlation of these changes with the bacterial flora.

EXPERIMENTAL PROCEDURE

Two types of muscle were studied, pork loin and pork trimmings, which are composed largely of flank muscle. In the first series of experiments the changes in the vitamin content of pork loins were determined.

Storage of Pork Loin: A few minutes after dispatch of the animals, seven U. S. Good pork carcasses were selected at random on the dressing floor of the pork-processing plant. That portion of the right loin between the seventh vertebra and 16th vertebra, inclusive, was removed from each

[1] Part of the material in this paper was presented before the Agricultural Food Division of the American Chemical Society at the New York meeting, September 11, 1944.

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animal and taken to the laboratory for assay as a control. The remainders of the carcasses were stored in the pork chilling cooler at 2°C.(36°F.) for three days, after which corresponding portions of the left loins were removed. Two of these were assayed immediately and the other five were stored in a cooler at 4°C.(40°F.). Two of these samples were assayed three days later and two more after 17 days in the cooler (six and 20 days after the start of the experiment). At the end of the 20-day period, which greatly exceeds the permissible storage for edible fresh pork, the loins smelled "sour" but were not moldy or soft. The last loin remained in the cooler for 65 days before analysis, becoming moldy and slightly rancid but not putrefied.

The longissimus dorsi muscle from each sample was trimmed free of fat and then ground three times through the three-mm. plate of an electric food hasher. After each grinding, the meat was thoroughly mixed. Since thiamin is of particular interest in pork, aliquots of the control loins (right) were assayed immediately for thiamin. The remaining portions were then sharp-frozen and stored for simultaneous assay with the experimental samples. Thiamin, riboflavin, niacin, and pantothenic acid determinations were made on the frozen control samples at the time of assay of the experimental loins. Thiamin analyses were made according to a modification of the thiochrome procedure of Hennessy and Cerecedo (1939) using a Coleman Model 11 electronic photofluorometer. Riboflavin, niacin, and pantothenic acid were determined by microbiological methods of Cheldelin, Eppright, Snell, and Guirard (1942).

As may be seen from the data (Table 1), except for niacin, there were no significant losses of vitamins. Sample 1 showed a slight loss of riboflavin and pantothenic acid after three days' storage, but since all other samples showed better retention after even longer storage, this sample must have been atypical.

Because decreases were found in the niacin content but not in the levels of the other vitamins, this experiment was repeated to make certain that the niacin data were typical. The results were in complete agreement with those of the first series (Table 1).

Storage of Pork Trimmings: In the second series of experiments, fresh lean pork trimmings were obtained directly from the pork cutting room, ground, and mixed thoroughly using clean but not sterile equipment. Portions of the meat were packed in pint jars and covered loosely with screw caps. A three-mm. hole was punched in each lid and plugged with a tuft of cotton to allow free passage of gases but to prevent further bacterial contamination. Before packaging, part of the meat was cured with a nitrate-nitrite-glucose formula. Samples of raw and cured meat were stored at —4°C.(24.8°F.) and at room temperature, 20-25°C.(68-77°F.), for the intervals indicated (Table 2).

Naturally, at room temperature the meat became "sour" within a few days and then putrefied. In four weeks the decomposition was so far advanced that expansion of the gaseous mass forced the lids from the jars.

Samples stored in the cooler remained in good condition for almost two weeks. Longer storage resulted in spoilage although the samples never became so completely decomposed as those stored at room temperature. The

TABLE 1

Retention of Vitamins in Pork Loins During Storage at 4°C. (40°F.)

Pair No.	Length of storage[1]	Vitamin content in micrograms per gram of protein											
		Thiamin			Riboflavin			Niacin			Pantothenic acid		
		Initial[2]	Final	Retained	Initial	Final	Retained	Initial	Final	Retained	Initial	Final	Retained
						First Storage Test							
	days			*pct.*			*pct.*			*pct.*			*pct.*
1	3	(57.2) 58.4	54.2	94	6.2	5.2	84	140	130	93	12.9	10.7	83
5	3	(83.4) 84.5	84.4	100	6.6	5.9	91	262	264	100	25.5	27.1	106
2	6	(76.4) 78.3	81.5	105	6.9	6.5	94	195	126	65	8.9	8.6	96
6	6	(77.3) 75.8	74.1	97	6.8	6.2	91	355	340	96	22.8	22.2	97
3	20	68.9 (69.4)	65.4	95	.....	.....	....	.....	.....	.....	.....	.....	.....
4	20	68.4 (68.7)	64.2	94	6.3	5.6	90	219	140	64	17.9	18.8	105
7	68	82.6	79.8	97	7.4	7.0	94	203	158	78	34.9	38.8	110
						Second Storage Test							
8	10	65.7	67.0	103	5.8	5.7	97	465	215	46	.....	.....	.....
9	10	72.5	72.7	100	6.5	6.4	98	388	292	75	.....	.....	.....
10	10	28.9	32.7	113	5.5	6.1	111	361	259	72	.....	.....	.....
11	10	28.5	30.9	108	7.4	7.2	97	171	111	65	.....	.....	.....
12	10	56.1	62.6	111	6.8	6.7	98	354	196	55	.....	.....	.....

[1] Storage during the first three days was at 2°C.. thereafter at 4°C. [2] Values in parentheses are from determinations made on fresh samples before freezing. The others are for same samples after freezing and storage for the same period as the experimental samples.

TABLE 2

Retention of Vitamins in Pork During Storage and Bacterial Spoilage

Length of storage	Temperature	Total bacterial count	Vitamin content in micrograms per gram of sample and as per cents of initial value							
			Thiamin		Riboflavin		Niacin		Pantothenic acid	
FRESH PORK										
days	*°C.*	*millions*	*µg./gm.*	*pct.*	*µg./gm.*	*pct.*	*µg./gm.*	*pct.*	*µg./gm.*	*pct.*
0	4	0.4	8.1	100	2.2	100	47.4	100	6.1	100
7	4	40	7.7	95	2.2	100	48.5	102	6.3	103
14	4	1,200	7.5	93	2.3	104	46.0	97	6.4	105
21	4	2,000	7.7	95	2.6	118	45.9	97	5.8	95
28	4	17,000	7.0	87	3.4	154	48.2	101	7.8	128
42	4	950	7.5	93	3.0	136	49.2	104	10.2	167
56	4	150	7.2	89	3.1	141	50.0	105	8.9	146
0	20-25	0.4	8.1	100	2.2	100	47.4	100	6.1	100
7	20-25	160	6.0	74	3.4	154	46.0	97	5.7	93
14	20-25	1,800	5.0	62	3.9	177	43.0	91	16.5	270
21	20-25	250	5.4	66	3.9	177	44.8	95	11.3	185
28	20-25	50	4.3	53	3.4	154	44.5	94	13.5	221
42	20-25	11	3.8	47	3.2	145	49.0	103	14.9	244
CURED PORK										
0	4	0.4	7.9	100	2.0	100	48.5	100	5.1	100
14	4	0.5	7.5	95	2.1	105	43.4	90	5.9	116
56	4	60	7.2	91	2.4	120	45.0	93	5.2	102
14	20-25	20	6.8	86	2.4	120	42.1	87	6.0	117

cured samples showed much better storage qualities, both in the cooler and at room temperature.

Portions of the fresh meat and each of the stored samples were taken for bacteriological study. Other portions were sharp-frozen and held at —29°C. (—20.2°F.) until the end of the storage period when they were assayed simultaneously to determine vitamin content. In this series of analyses the fluorometric riboflavin procedure of Peterson, Brady, and Shaw (1943) was used as a check against the microbiological method. Excellent agreement was obtained for all samples except the ones showing very high bacterial counts. In those cases the extracts were turbid, thus preventing satisfactory analysis by the fluorometric method (Table 2).

At 4°C. there were no significant changes in the content of any of the vitamins during two weeks' storage. Upon longer storage, there were slow losses of thiamin but there were increases of riboflavin and pantothenic acid. Niacin remained unchanged in concentration. Samples stored at room temperature responded similarly, but the changes occurred more quickly and to a greater extent. In no case did a significant change occur in the vitamin content of the sample before bacterial spoilage had advanced to a stage such that the meat was entirely unfit for use as food.

TABLE 3

Changes in Bacterial Flora of Pork Upon Storage

Temperature	Storage	Total count		Aerobic differential count		
		Aerobic forms	Anaerobic spores	*Pseudomonas*	*Achromobacter*	Others
°C.	*days*	*millions/gm.*	*No./gm.*	*pct.*	*pct.*	*pct.*
Fresh	....	0.38	10	10	70	20[1]
20-25	7	160	1,000	44	55	1
20-25	14	1,800	10,000	100	0	0
20-25	21	250	1,000	90	5	5
20-25	28	50	100	80	0	20[2]
20-25	42	11	1,000,000	....	....	....[3]
4	7	40	10	29	70	1
4	14	1,200	100	75	25	0
4	21	2,000	100	80	20	0
4	28	17,000	10,000	100	....	....
4	42	950	1,000	90	1	9
4	56	150	1,000	100	....	....
Cured						
20-25	14	20	0	....	....	....[3]
4	14	0.55	100	10	80	10
4	56	60	100	100	0	0

[1] Twenty micrococci. [2] Ten mold, 10 proteus. [3] All pin-point colonies.

Bacterial populations of the samples were determined using plain agar plates. Naturally, all forms of bacteria do not respond equally well to such conditions, but previous experience has shown that this medium is adequate for the most common meat flora. Determinations of the principal genera were made but no effort was made to define species. Bacteria of the genus *Pseudomonas* gradually overgrow the other types so that the final population was almost exclusively of that genus (Table 3).

DISCUSSION

It is apparent that losses of thiamin from pork during customary cooler storages are negligible. At room temperatures losses occur, but these can be of only theoretical interest since pork held under such conditions spoils in a few hours. Decreases in the thiamin content of these samples, however, make it seem probable that the slight losses observed for the meat stored in the cooler are real and not due to experimental errors. With the present data it is impossible to determine whether the losses are due to hydrolytic breakdown of the thiamin or to its utilization by bacteria. The slow decomposition in samples stored at 4°C. might be an indication that bacteria are not involved, since large bacterial populations were found for these samples.

As with thiamin, storage for short periods at 4°C. does not cause changes exceeding five to 10 per cent in the concentration of riboflavin either in loin or ground pork. The increase of riboflavin during the later stages of the ground-pork-storage experiments, both at cooler and room temperatures, is probably due to microbial synthesis since increases in riboflavin roughly parallel increases in the bacterial flora. The failure of loin No. 7 to show this increase may be explained on the basis that the bacterial contamination was not great despite its long storage. Although the surface of this loin was moldy and slightly rancid, the longissimus dorsi muscle had no off-odor. Apparently the covering of fat and bone had protected this muscle from bacterial attack. Unfortunately a bacterial count was not obtained for this sample. The small riboflavin increases in the cured samples may be attributed to the slow bacterial growth in the presence of the cure.

With niacin the picture is less clear since three loins showed excellent retention while the others lost amounts varying up to 54 per cent of the amount originally present. With ground pork trimmings, where all samples were uniform at the start, niacin was almost completely retained. Many other studies, mainly of cured or canned meats, indicate 90 to 100 per cent retention of niacin on storage at 4°C. for periods up to 30 days. It is possible, as pointed out by Schweigert, McIntire, and Elvehjem (1944), that variations in the niacin content of paired loins is sufficiently great to account for the differences. This seems rather improbable, however, as all of the questionable retentions are lower than 100 per cent, whereas by normal distribution at least one high value should have been found. Furthermore, the thiamin, niacin, and riboflavin contents of eight sets of companion muscles (two beef and six pork, including loin) have been determined and in no case have deviations of more than 10 per cent been found. On the basis of the present data it seems either that niacin is more stable in flank muscle than in loin muscle, or that it is more stable in ground meat than in the whole muscle. This aspect of the problem is being studied further.

Pantothenic acid closely parallels riboflavin in its response to storage, apparently increasing markedly owing to bacterial synthesis in high-count samples.

It is interesting to note that the greatest number of bacteria were found in the samples stored at 4°C. although the rate of increase of

population was much slower than at room temperature. The riboflavin and pantothenic acid levels reached greater maxima and reached these more quickly at higher temperatures regardless of the lower total number of bacteria found at any given time in these samples. Perhaps the increase in these vitamins is proportional to the metabolic rates of the bacteria rather than to the numbers present. It should be borne in mind that the bacterial counts give only the number of viable bacteria, and do not give any indication of the total number of bacteria which have existed at one time or another in the meat. If "cumulative" populations could be determined they might parallel the change in vitamin content more closely.

SUMMARY

Experiments have been conducted to determine the stability of thiamin, riboflavin, niacin, and pantothenic acid in pork loins and in ground pork flank muscles during storage.

During periods of less than two weeks at a temperature of 4°C. losses of thiamin, riboflavin, and pantothenic acid were less than 10 per cent in either type of tissue. As much as 54 per cent of the niacin in loins was lost, but this vitamin remained at a constant level for as long as 56 days in ground flank muscle.

In the case of loins, prolongation of the storage period at 4°C. to 68 days did not result in changes significantly different from those for the shorter period. With ground pork at 4°C., however, there was a gradual decrease in thiamin during 56 days to 89 per cent of the initial value. Riboflavin and pantothenic acid increased to values over 150 per cent of the original, apparently owing to microbial synthesis. Niacin remained relatively constant.

At room temperature samples of ground pork quickly putrefied. Thiamin decreased gradually to 47 per cent of the initial level, but riboflavin and pantothenic acid increased to levels as high as 177 and 270 per cent, respectively. Niacin remained relatively constant.

Cured pork showed much slower bacterial growth and correspondingly smaller changes in riboflavin and pantothenic acid. The losses of thiamin and niacin compared favorably with those for the uncured meat.

Except for the loss of some niacin, the vitamin content of pork remained unchanged during storage up to a point at which spoilage is noticeable.

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INVESTIGATION OF *SALMONELLA* CONTENT OF POWDERED WHOLE EGG WITH NOT MORE THAN TWO PER CENT MOISTURE CONTENT

II. GENERAL SURVEY ON OCCURRENCE OF SPECIES OF *SALMONELLA* IN HIGH-QUALITY EGG POWDER

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Data are being accumulated on the occurrence of pathogenic bacteria in egg and egg products which tend to show that these products may be important as vehicles for the dissemination of communicable diseases.

Pathogenic hemolytic streptococci have been isolated from eggs, Trossarelli and Massano (1938) and Schneider (1944). As might be expected, *Salmonella* types, including *S. pullorum*, which are pathogenic for man—Edwards and Bruner (1943)—as well as fowls—Rettger (1909), Tittsler, Heywang, and Charles (1928), and Hinshaw, McNeil, and Taylor (1944)—are not infrequently isolated from eggs—Tittsler, Heywang, and Charles (1928), Gibbons and Moore (1944a,b), and Seligman, Saphra, and Wasserman (1943).

A large amount of spray-dried whole egg is being produced for use by the Armed Forces of the United States, hence microbiological studies are of interest from the standpoint of health. The standards adopted for the production of powdered whole egg for the Armed Forces as defined, at first, in the Quartermaster Tentative Specification, CQD No. 117A, 28 Jan. 44, and later in CQD No. 117B, 12 Dec. 44, are in line with the steps which are being taken to control the occurrence of pathogens; namely, under Veterinary Corps supervision, Army requirements in sanitary and operational facilities are closely observed which permit the rigid selection of raw material of high quality. This includes specific microbiological requirements on the raw whole egg as well as the bacteriological and chemical grading of the whole-egg powder. The current Army requirements in processing egg powder of low moisture content suggested the investigation of the *Salmonella* content and the potential health hazard associated with this quality product. To obtain a low moisture-whole egg powder the preheating of the liquid whole egg prior to multistage drying is advantageous, Stateler (1945). The preheater raises the temperature of the liquid egg to approximately 61°C.(142°F.) prior to spray drying.

This report includes a general survey of 901 powdered whole-egg lots picked at random.

EXPERIMENTAL PROCEDURE

Routinely, three-pound cans of egg powder were submitted to the laboratory for chemical and bacteriological analyses. Under aseptic conditions

[1] From the Seventh Service Command medical laboratory.

egg powder was removed to wide-mouth, 240-ml. screw-top, glass jars. Each aliquot examined represented approximately a 3,600-pound lot. The interim between production and examination ranged from one to seven days.

Five grams of egg powder were weighed on sterile, smooth paper, transferred to a 120-ml., wide-mouth, glass-stoppered bottle containing 45 ml. of sterile distilled water and a teaspoonful of glass beads, and then agitated in a mechanical shaker for five minutes. Twenty-five ml. of the egg suspension was inoculated into 25 ml. of double-strength Selenite F enrichment medium (B-B-L) and incubated 18 to 24 hours at 37°C.(98.6°F.).[2] S. S. (Difco) and desoxycholate citrate (B-B-L) agars were the differential plating media used. Plates of each were streaked from the enrichment broth and incubated 18 to 24 hours at 37°C. If no growth was observed, plates were incubated for an additional 24 hours.

A minimum of 10 characteristic colonies was picked to Kligler's iron agar (Difco) modified by the addition of one per cent sucrose. Cultures showing an acid butt with or without gas, or production of hydrogen sulfide, and an alkaline slant after 24 hours' incubation at 37°C. were inoculated into four ml. of nutrient broth. After incubation for three to four hours at 37°C., cultures were examined for Gram-negative and motile organisms. Presumptive *Salmonella* organisms were then inoculated into the following carbohydrate broths: dextrose, sucrose, maltose, lactose, mannitol, xylose, glycerol, dulcitol, sorbitol, arabinose, inositol, salicin, raffinose, trehalose, rhamnose; also into the following differential media: gelatine, milk, tryptone broth, nitrate agar, Clark-Lubs broth, Simmon's citrate, and Jordan and Harmon's phenol red tartrate agar. The specific agglutinating sera used for serological typing were supplied by the Army Medical Center, Washington, D. C. Identity of random cultures was confirmed by the Army Medical Center.

RESULTS

Salmonella organisms were isolated from 32 lots, or 3.18 per cent. Five types were encountered: *S. oranienburg*, *S. tennessee*, *S. oregon*, *S. montevideo*, and *S. pullorum*. Excluding *S. pullorum* the incidence of *Salmonella* in 901 samples of high-quality egg powder was 0.67 per cent.

Of 10 plants involved in the survey (Table 1) *Salmonella* types were found in egg powder from all except Plants 5, 9, and 10. With exception of 103 lots from plant No. 7 and one experimental run from plant No. 2, all plants used preheaters. The largest percentage of *Salmonella* organisms were isolated from plant No. 7.

It was observed (Table 2) that the occurrence of *Salmonella* in plants using both preheaters and multistage drying was 1.63 per cent of 797 lots examined; while 104 lots produced without the aid of a preheater showed an incidence of 18.27 per cent. The latter observation was higher than Gibbons and Moore's (1944a) report of 7.37 per cent of 328 lots examined. Whether or not preheaters were used in the latter study is not known.

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[2] Tetrathionate broth base, as an enrichment medium, was employed in a similar manner for a short interval.

TABLE 1

General Survey on Occurrence of Salmonella in Egg Powder From Ten Plants Operating in the Seventh Service Command

Plant No.	Total lots examined	Number and per cent isolation of *Salmonella*
1	121	3 or 2.48
2	79	1[1] or 1.27
3	74	1 or 1.35
4	72	4 or 5.55
5	91	0
6	160	3 or 1.88
7	17[2]	0
	103[3]	18 or 17.48
8	108	2 or 1.85
9	74	0
10	2	0
Grand total	901	32 or 3.18

[1] Experimental run—preheater not used. [2] Processed with the aid of a preheater. [3] Processed without the aid of a preheater.

TABLE 2

General Survey—Use of Preheaters

Treatment	Total lots examined	Number and per cent isolation of *Salmonella*
Preheaters used	797	13 or 1.63
Preheaters not used	104	19 or 18.27

TABLE 3

Monthly Distribution of Salmonella

Date	Total lots examined	Number and per cent isolation of *Salmonella*
October, 1944	341	4 or 1.17
November, 1944	286	5 or 1.75
December, 1944	211	22 or 10.43
January, 1945	63	1 or 1.59

TABLE 4

Salmonella Types Isolated

Type	Number of times isolated	Per cent of total
S. pullorum	26	81.25
S. tennessee	2	6.25
S. oranienburg	2	6.25
S. oregon	1	3.12
S. montevideo	1	3.12

Tables 3 and 4 are self-explanatory; Table 5 shows comparison of tetrathionate broth base (Difco) with Selenite F enrichment (B-B-L) for isolation of *S. pullorum.*

TABLE 5

Isolation of S. pullorum From Duplicate Samples of Powdered Egg

Enrichment medium	Total examined	Number and per cent isolation of *S. pullorum*
Tetrathionate broth base	61	0
Selenite F	61	15 or 24.59

DISCUSSION

Salmonella organisms when present in egg powder, are few in number, Gibbons and Moore (1944a). For this reason direct isolations on differential plating media are difficult. In this study double-strength preparations of enrichment media were diluted to single strength by the addition of an equal volume of egg suspension. Gibbons and Moore reported tetrathionate broth base as a satisfactory enrichment medium. In the isolation of *S. pullorum* from powdered egg, one observation (Table 5) favored Selenite F enrichment medium.

A comparison of our data with those of Gibbons and Moore (1944a) shows that, of a total of 14 *Salmonella* types encountered in powdered egg, 10 (Gibbons and Moore, 6; Schneider, 4) belong to Group C of the Kauffman-White schema which rank second as the most prevalent *Salmonella* species in American surveys of infections in man, Seligman, Saphra, and Wassermann (1943). Prevalence in the United States of these *Salmonella* types is 33 to 36 per cent of the total surveyed by Seligman, Saphra, and Wasserman (1943). The data of Edwards and Bruner (1943), Edwards (1939), and Hinshaw *et al.* (1944) indicate that many outbreaks in avians have been caused by Group C *Salmonella* types. Hinshaw *et al.* (1944) speak of ". . . . spread and perpetuation of *Salmonellosis* by transmission of the infection through the egg. Egg transmission has been proven for several types . . . ." The isolation of Group C organisms from powdered egg strongly suggests poultry to be the most common source of the organisms in the powder.

A report of *S. tennessee* from a source other than human was recently made by Schneider (1945). *S. oregon* from Edwards and Bruner (1943) and *S. montevideo*—Edwards and Bruner (1943), Hinshaw *et al.* (1944), Hormaeche, Surraco, Pelutto, and Aleppo (1943) and Edwards (1939)—previously associated with poultry and human outbreaks are here isolated from powdered egg.

As to the public-health significance sufficient data are not available to incriminate powdered egg in food-poisoning outbreaks. Gibbons and Moore (1944b) regard the danger of contamination from well-cooked food as slight. On the other hand, they go on to state that "the finding in man of *S. pullorum* (2) and other types of *Salmonella* common to fowl indicates that infection can occur from birds and the possibility of transmission by

eggs and egg products must be considered. Reconstituted eggs might be consumed raw, as in eggnog, and if made from contaminated powder and an incubation period were allowed between reconstitution and consumption, there is a possibility of infection."

The present survey shows that current Army requirements in the production of whole eggs effect a marked reduction of the *Salmonella* content of the whole-egg powder and, therefore, lessen the potential health hazard to consumers. In particular, it appears that the preheater, which was introduced to lower the moisture content of whole-egg powder, Stateler (1945), has an additional effect of reducing the *Salmonella* content in whole-egg powder.

A study is at present in progress, investigating in more detail the effect of the preheater on the *Salmonella* content of powdered egg.

SUMMARY

Nine hundred and one samples of high-quality powdered egg were examined, from which 32, or 3.18 per cent, isolations of *Salmonella* organisms of several species were made.

Of 797 lots processed with the aid of both preheater and multistage drying, *Salmonella* organisms were isolated from 13, or 1.63 per cent.

Of 104 lots spray dried without the aid of a preheater, *Salmonella* species were isolated from 19, or 18.27 per cent.

Five *Salmonella* types were encountered in the survey: *S. pullorum, S. oranienburg, S. tennessee, S. oregon, and S. montevideo.*

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[3] From the Seventh Service Command Medical Laboratory.

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EFFECT OF INSTITUTIONAL COOKING METHODS ON VITAMIN CONTENT OF FOODS

II. ASCORBIC ACID CONTENT OF POTATOES[1]

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The potato has been analyzed for its ascorbic acid content by numerous investigators; as a result, considerable information has been accumulated relative to varietal differences and to various cooking procedures used on a family or laboratory scale. At the time this study was undertaken, however, data had not been reported concerning the effect of large-quantity cookery methods. This paper is a report of such a study, undertaken in October, 1942. The foods were obtained at the College cafeteria which, at the beginning of the study, served approximately 500 students and which, six months later, served 750 Army Air Corps students. The data indicate that from 67 to 88 per cent of the ascorbic acid originally present in the potato was destroyed during the interval between the paring and serving of the potatoes.

EXPERIMENTAL PROCEDURE

The Green Mountain potatoes used in this study were from the same cooking lots used in the previously reported study on the thiamin content of potatoes as influenced by institutional cooking methods by Wertz and Weir (1944). Reference to that paper should be made for detailed information concerning preparation of the potatoes for serving.

Sampling of Potatoes: The potatoes were sampled at the following steps in preparation: (1) pared, (2) soaked six hours in tap water, (3) steamed at 107.2°C.(225°F.) for one hour, (4) mashed, (5) steamed and held for one and one-half hours at 69.4°C.(157°F.), (6) mashed and held one and one-half hours at 157°F. For the analysis of the whole potato, either raw or steamed, 12 to 16 potatoes were selected from different areas in the bags or the steam trays. Wedge-shaped slices were cut from the bud end to the stem end of the potato and immediately dropped into a tared flask containing a freshly prepared extracting mixture. The mashed potatoes were sampled immediately upon cessation of the beater by removing small portions from various areas in the vat until 600 to 700 grams were obtained, these portions being quickly mixed before samples were transferred to the tared flasks. Fifteen- to 20-gram samples of either the raw or the cooked potatoes were used for each determination.

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[1] Contribution No. 525 of the Massachusetts Agricultural Experiment Station.

[2] A portion of these data were submitted in thesis form by C. Edith Weir in partial fulfillment for the degree of Master of Science at Massachusetts State College, June, 1943.

Extraction and Determination of Ascorbic Acid: The methods for extraction and titration of ascorbic acid were essentially those of Bessey and King (1933) as modified by Mack and Tressler (1937). A mixture of two per cent H_3PO_4 and five per cent H_2SO_4 was used as the extracting fluid. Care was taken that the potato samples were completely covered with the solvent throughout the entire process of triturating with sand, this process being under way within five minutes after the samples were obtained. Separation of the extract from the potato tissue was effected by centrifugation. Freshly boiled, redistilled water was used in making the extracts to volume, in diluting the samples, and in preparing the solution of 2,6 dichlorophenolindophenol. The standardized dye was run into the aliquots from a burette graduated to 0.01 ml.

At the beginning of the study an attempt was made to determine dehydroascorbic acid in potatoes at different stages of preparation. A three-per cent metaphosphoric acid extract of the potatoes buffered to pH 3.5-3.7, as recommended by Bessey (1938), was used; hydrogen sulfide was bubbled through the extract for 30 minutes. Following the subsequent removal of the sulfide an increase in the reducing properties of the potato extracts was found. This increase, however, was no greater than that observed in portions of buffered acid devoid of potato extract. Since this procedure failed to reveal the presence of dehydroascorbic acid in the potatoes at any stage of preparation, it was abandoned. The term ascorbic acid, as used in this paper, therefore, refers to the reduced form only.

RESULTS

Ascorbic Acid Content of Raw Potatoes: Potatoes in the raw condition were analyzed for ascorbic acid on 22 different occasions, the analyses being conducted over the seven-month interval between November, 1942, and May, 1943. At the beginning of the study the potatoes contained 25.6 mg. ascorbic acid per 100 grams, whereas in the following May they contained only 7.3 mg. The average value for the 22 determinations was 12.1 mg.

Effect of Soaking: The data relative to the effect of holding the potato in water for a period of six hours (Table 1) show that the changes in ascorbic acid observed in the 10 trials ranged from +15.9 to —16.8 per cent and averaged —2.9 per cent. Statistical odds of 1 to 1, Student (1925), indicated that the differences were not significant.

Effect of Steam-Cooking: The effect of steam-cooking on the ascorbic acid content of eight lots of potatoes (Table 2) shows that the values for the raw potatoes, which ranged from 17.0 to 7.5 mg. per 100 grams, decreased to 9.0 to 2.6 mg. per 100 grams for the steam-cooked potatoes. The percentage loss in ascorbic acid computed on a dry-weight basis, ranged from 33 to 68 per cent and averaged 55 per cent. Six additional lots, Lots 1 to 14 (Table 3), show similar losses owing to steam-cooking. Whereas the values ranged from 23.6 to 7.3 mg. per 100 grams in the raw potato, they decreased to 12.1 to 2.4 mg. per 100 grams following steam-cooking, the percentage losses ranging from 37 to 67 and averaging 54 per cent. The weighted average for the steam-cooking losses in ascorbic acid in these 14 lots of potatoes was 54.4 per cent.

Effect of Mashing: During the mashing process 10 lots of steam-cooked potatoes lost from 25 to 74 per cent of the ascorbic acid present in the potato at the end of the cooking period or from 12 to 31 per cent of that present in the raw, pared potato (Table 3). The ascorbic acid values for the mashed potatoes ranged from 0.8 to 9.0 mg. per 100 grams and averaged 2.7 mg.

TABLE 1

Change in Ascorbic Acid Content of Potatoes Held in Water for Six Hours

Lot No.	Raw, pared			Raw, pared, soaked			Difference between raw and soaked
	Moisture	Ascorbic acid		Moisture	Ascorbic acid		
		As examined	Dry weight		As examined	Dry weight	
	pct.	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*
2	82.0	16.0	88.9	81.4	17.0	91.4	+2.8
3	82.0	12.3	68.3	81.4	13.5	72.6	+6.3
4	82.4	19.0	108.0	81.4	19.0	102.2	−5.4
6	82.4	14.5	82.4	84.3	15.0	95.5	+15.9
7	84.2	11.6	73.4	83.0	11.6	68.2	−7.1
10	81.2	14.6	77.6	80.8	12.4	64.6	−16.8
20	80.7	25.6	132.6	82.3	19.7	111.3	−16.1
21	78.6	10.9	50.9	81.4	10.3	55.4	+8.8
22	81.7	7.9	43.2	77.8	8.7	39.2	−9.3
23	79.0	10.7	51.0	82.0	8.4	46.9	−8.0
Av	81.4	14.3	77.6	81.6	13.6	74.7	−2.9

Effect of Holding in a Steam Oven: An additional loss in ascorbic acid was found to occur when the potatoes were held in a steam oven prior to serving (Tables 2 and 3). For the eight lots of potatoes served whole following this holding period, the ascorbic acid content varied from 1.7 to 8.0 mg. per 100 grams, the mean value being 3.9. The destruction of this vitamin averaged 27 per cent of that left in the potato at the end of cooking or 10 per cent of that in the raw, pared potato. In the 10 lots of mashed potatoes the loss owing to holding in a steam oven averaged 32 per cent. The ascorbic acid content of these mashed potatoes averaged only 1.6 mg. per 100 grams.

DISCUSSION

The data relative to the soaking of potatoes, a procedure commonly used in homes and in institutions, do not indicate a lowering in ascorbic acid value. Lauersen and Orth (1942) have reported only negligible losses in potatoes pared, cut in pieces, and soaked in water for 24 hours. Under the conditions used by Richardson and Mayfield (1943) potatoes pared, cut, and soaked four hours in fresh water suffered a four to five per cent loss owing to soaking. The combined data suggest that, so far as ascorbic acid is concerned, the practice of paring potatoes and holding them in water for several hours before cooking is not detrimental.

The cooking of the potatoes by means of low-pressure steam was accompanied by an average loss in ascorbic acid of 54 per cent. Heller, McCay, and Lyon (1943) likewise found substantial losses, 50 and 91 per cent

TABLE 2

Losses in Ascorbic Acid in Potatoes Owing to Steam-Cooking and to Holding, in a Whole Condition, in a Steam Oven

Lot No.	Raw, pared			Steamed 1 hr. at 107.2°C.(225°F.)				Held in steam oven 1½ hr. at 69.4°C.(157°F.)				Total loss[2]
	Moisture	Ascorbic acid		Moisture	Ascorbic acid			Moisture	Ascorbic acid			
		As examined	Dry weight		As examined	Dry weight	Loss		As examined	Dry weight	Loss[1]	
	pct.	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*pct.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*pct.*
2	81.4	17.0	91.4	80.8	9.0	46.9	48.7	80.6	8.0	41.2	12.1	54.9
11	81.8	13.0	71.4	79.5	9.8	47.8	33.0	79.4	7.7	37.4	21.7	47.6
13	79.9	9.2	45.8	77.3	4.7	20.7	54.8	76.4	3.8	16.1	22.2	64.8
15	79.9	8.2	40.8	77.2	3.2	14.0	65.7	75.7	2.4	9.9	29.3	75.7
16	80.7	8.6	44.6	79.7	3.7	18.2	59.2	78.8	1.8	8.5	53.3	80.9
17	79.9	7.5	37.3	78.9	2.6	12.3	67.0	78.6	1.9	8.9	27.6	76.1
18	79.9	7.5	37.3	78.8	4.4	20.8	44.2	78.5	3.6	16.7	19.7	55.2
19	80.3	7.6	38.6	79.2	2.6	12.5	67.6	79.7	1.7	8.4	32.8	78.2
Av	80.5	9.8	50.9	78.9	5.0	24.1	55.0	78.5	3.9	18.4	27.3	66.7

[1] Computed on the basis of ascorbic acid in the steam-cooked potato. [2] Computed on the basis of ascorbic acid in the raw, pared potatoes.

TABLE 3

Losses in Ascorbic Acid in Potatoes Owing to Steam-Cooking, Mashing, and Holding in a Steam Oven

Lot No.	Raw, pared			Steamed 1 hr. at 107.2°C.(225°F.)				Mashed				Held in steam oven 1½ hr. at 69.4°C.(157°F.)				Total loss[3]
	Moisture	Ascorbic acid		Moisture	Ascorbic acid			Moisture	Ascorbic acid			Moisture	Ascorbic acid			
		As examined	Dry weight		As examined	Dry weight	Loss		As examined	Dry weight	Loss[1]		As examined	Dry weight	Difference[2]	
	pct.	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*pct.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*pct.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*pct.*
1	82.0	23.6	131.1	80.8	12.1	63.0	51.9	80.9	9.0	47.1	25.2	80.6	5.2	26.8	—43.1	79.6
5	82.0	9.2	51.1	81.4	3.4	18.3	64.2	80.9	2.0	10.4	43.2	80.6	1.4	7.2	—30.8	85.9
8	82.4	11.4	64.8	81.4	7.6	40.9	36.9	80.9	4.6	24.1	41.1	79.5	2.4	11.7	—51.4	81.9
10	81.2	14.6	77.6	80.2	7.4	37.4	51.8	80.6	4.2	21.6	42.2	80.5	1.2	6.2	—71.3	92.0
12	83.4	9.8	59.0	79.8	5.9	29.2	50.5	78.8	1.6	7.5	74.3	78.0	0.8	3.6	—52.0	93.9
14	78.6	7.3	34.1	78.8	2.4	11.3	66.9	78.0	0.8	3.6	68.1	78.8	0.6	2.8	—22.2	91.8
15[4]	79.9	8.2	40.8	77.2	3.2	14.0	65.7	78.8	1.7	8.0	42.8	76.9	1.4	6.1	—23.8	85.0
16[4]	80.7	8.6	44.6	79.7	3.7	18.2	59.2	77.5	1.1	4.9	73.1	79.3	1.2	5.8	18.4	87.0
17[4]	79.9	7.5	37.3	78.9	2.6	12.3	67.0	78.2	1.1	5.0	59.3	78.2	0.9	4.1	—18.0	89.0
19[4]	80.3	7.6	38.6	79.2	2.6	12.5	67.6	78.9	1.2	5.7	54.4	78.9	0.8	3.8	—33.3	90.0
Av.	81.0	10.8	57.9	79.7	5.1	25.7	58.2	79.4	2.7	13.8	52.4	79.1	1.6	7.8	—32.7	87.6

[1] Computed on the basis of ascorbic acid in the steam-cooked potatoes. [2] Computed on the basis of ascorbic acid in the mashed potatoes. [3] Computed on basis of ascorbic acid in the raw, pared potatoes. [4] Lots 15, 16, 17, and 19 also appear in Table 2.

respectively, in two lots of steam-cooked potatoes. The study by Daum, Aimone, and Hollister (1943) of eight analyses of new potatoes and 30 analyses of old potatoes indicated a loss owing to cooking of only seven and 10 per cent for the new and old potatoes, respectively; unfortunately, the method of cooking and the quantity of potatoes used were not specified.

The destructive effect of the mashing process as carried out under these institutional conditions is evident. Approximately one-half of the ascorbic acid remaining in the potato at the end of cooking was destroyed; a portion of this decrease, not exceeding 10 per cent, could be explained by the addition to the potatoes of milk of lower ascorbic acid value. Roberts and Roberts (1942), assaying mashed potatoes served in an institution, reported that "all the vitamin C was destroyed" in potatoes well aerated in an electric mixer and held on a steam table for a considerable period. Kahn and Halliday (1944) found only a 39-per cent loss in potatoes, pared, cut in halves, steamed, and then mashed. Jensen (1943) reported that the effect of mashing on the ascorbic acid content of potatoes was not immediate but that the rate of loss during subsequent heating was considerably accelerated. Lyons and Fellers (1939) found little difference between boiled and mashed potatoes prepared in small quantities.

The holding of the potatoes in a steam warming oven for one and one-half hours after cooking is likewise attended by significant losses in ascorbic acid; in this study the losses in the whole potato and in the mashed potatoes, respectively, approximated 27 and 33 per cent of the vitamin present at the beginning of the holding period. This value is considerably lower than the 56-per cent loss reported by Kahn and Halliday for mashed potatoes held for a 75-minute period and the 60-per cent loss observed by Daum and co-workers in potatoes held 60 minutes on a steam table.

The well-known deteriorating effect of storage of potatoes on their ascorbic acid value is seen in this study. The new fall potatoes examined in November contained 25.6 mg. per 100 grams, whereas in May they contained less than one-third of that amount.

Primarily because of its richness in ascorbic acid, the potato has assumed a position of importance in dietetics. This study, however, clearly shows that the methods of cookery used in some institutions may destroy almost all of this vitamin. This is particularly true for the mashed potato, held in the steam oven for as long as one and one-half hours following the mashing; in eight of the 10 trials the ascorbic acid content of the mashed potatoes did not exceed 1.4 mg. per 100 grams. Based on the average value of 1.6 mg. per 100 grams for the 10 determinations in this study, the quantity of ascorbic acid ingested by an individual consuming these potatoes in the amounts prescribed by the Quartermaster Corps of the United States Army, Howe (1942), i.e., 0.69 pound per day, would have approximated five mg. or one-fifteenth of the suggested daily allowance, National Research Council (1943). Obviously the individual who consumes little more than an average-sized portion of potatoes mashed under the conditions inherent in this study should almost entirely discount the potato as a source of ascorbic acid. Likewise the whole potatoes, steam-cooked and held one and one-half hours before serving, would not have contributed a significant quantity of this vitamin; a pound of these potatoes daily would

have been required to supply even one-tenth of a man's daily allowance. On the other hand, one pound of steam-cooked potatoes would have contributed as much as 22 mg. had they been eaten immediately at the cessation of cooking. It is hoped that future studies in this field will investigate methods of institutional cookery which will be more conducive to the conservation of the ascorbic acid in potatoes than were the methods used in this study.

SUMMARY

Green Mountain potatoes were analyzed for reduced ascorbic acid at the following stages in preparation: (1) pared, (2) soaked six hours in water, (3) steamed at 225°F. for one hour, (4) mashed, (5) steamed and held for one and one-half hours at 157°F., and (6) mashed and held one and one-half hours at 157°F. The average values for ascorbic acid in the potatoes at these stages of preparation were 12.1, 13.6, 5.6, 2.7, 3.9, and 1.6 mg. per 100 grams, respectively. Soaking appeared to have little, if any, effect on the ascorbic acid. Fourteen lots of potatoes subjected to steam-cooking showed losses in ascorbic acid which ranged from 33 to 68 per cent and averaged 54.4 per cent. The mashing process was accompanied by a loss of 52 per cent of the ascorbic acid in the cooked potato. On being held one and one-half hours in a steam warming oven whole, cooked potatoes lost 27 per cent and mashed potatoes lost 33 per cent of their remaining ascorbic acid. The over-all destruction of the vitamin approximated 67 per cent for the whole potatoes and 88 per cent for mashed potatoes.

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EFFECT OF CERTAIN ENVIRONMENTAL CONDITIONS ON HEAT RESISTANCE OF MICROCOCCI

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In a study of the heat resistance of bacterial spores in fatty substrates some incidental micrococci were observed at times to survive the destruction of the test spores. Preliminary studies indicated that this anomalous finding was not due to inherent resistance of the micrococci, but rather to a protection afforded by the nature of the heating substrate. As a result, an investigation was made to determine the effect of certain environmental conditions on the heat resistance of these micrococci. The literature supplies only meager quantitative information on the subject of abnormal resistance to heat by nonsporing bacteria. Gay, Atkins, and Holden (1931) have reported the case of a dried *Pneumococcus* which, when heated under dry conditions, survived 30 minutes but not 60 minutes at 110°C.(230°F.). The same *Pneumococcus* failed to survive 60 minutes at 56°C.(132.8°F.) in the moist state. Ruyle and Tanner (1935), Savage, Hunwicke, and Calder (1922), and others have reported the isolation of micrococci in certain instances from sound canned meats of high fat content which had been processed at times and temperatures adequate to destroy resistant spoilage types. Jensen (1942) has conducted heat resistance tests on these micrococci in water and oil in test tubes. He states that it has been shown both in practice and theory that the heat resistance of certain nonresistant cocci is greatly increased when oils or fats are used as the suspending menstrua. The term micrococci is used in this paper to conform to the terminology of previous investigators. It refers to Gram-positive cocci, such as staphylococci, streptococci, diplococci, etc., which are natural contaminants encountered in meat packing. There is no evidence that these micrococci grow into the canned product and evidence thus far acquired supports the belief that the same factor or factors which act to protect the organisms also act to prevent their subsequent growth.

In the present study, therefore, emphasis was placed on the effect of environmental conditions, such as the presence or absence of moisture and of fat, on the resistance of nonsporing bacteria. The moist resistance of a sporeforming putrefactive anaerobe, NCA No. 3679, which was the test organism in the original study, and that of a highly resistant, flat-sour organism, NCA No. 1503, were determined for comparison.

EXPERIMENTAL PROCEDURE

Cultures of micrococci isolated from heat-processed, fatty products were prepared on 0.5 per cent dextrose nutrient agar. After 24 hours' incubation at 37°C.(98.6°F.) the growth was taken up in saline, centrifuged, and washed. One portion was prepared in a heavy aqueous suspension and another was reduced to the dry state. Both lots were standardized as to

total count. The dry state was obtained according to the method of Gay, Atkins, and Holden (1931) by desiccating the concentrated bacteria over calcium chloride *in vacuo*. The dried bacteria, before being used for heat-resistance determinations, were tested for dryness by the following methods:

1. Anhydrous alcohol-anhydrous copper sulfate method.
2. Heating with anhydrous copper sulfate in capillary tubes.
3. Heating and rapidly chilling the bacteria in sealed capillary tubes to detect condensed moisture.

Heat-resistance tests were made on the moist and dried bacteria in the presence of moisture, as in broth and moist fat, and on dried bacteria in the absence of moisture, as in anhydrous fat and in air. The moist fat consisted of unsalted butter containing about 15 per cent moisture, and the anhydrous fat consisted of butter which was dried at 180°C.(356°F.) until no moisture could be detected by the anhydrous alcohol-anhydrous copper sulfate method. The heat-resistance tests were performed generally according to the method of Bigelow and Esty (1920) using thermal death time tubes of seven mm. inside diameter with one mm. wall in a three to five tube series as used by Esty and Williams (1924). For dry bacteria heated in air, capillary tubes of various sizes were used in addition to the thermal death time tubes, in order to check a possible insulating effect of the air. All tubes were sterilized before use, and those used for testing under dry conditions were stored in a 55°C.(131°F.) incubator.

The resistance of the putrefactive anaerobe, NCA No. 3679, and that of the flat-sour organism, NCA No. 1503, was determined in neutral phosphate in the thermal death time tubes. The spores of the putrefactive anaerobe were obtained from an aqueous stock spore suspension, prepared about one and one-half years previously, from a centrifuged pork-liver broth culture. The spore suspension of the flat-sour organism was prepared from a yeast extract agar culture after a 48-hour incubation at 55°C. The spores were washed and suspended in distilled water.

The heating of the charged tubes was done either in a constant-temperature water or oil bath at selected temperatures. After heating, the thermal death time tubes were subcultured into a suitable nutrient broth or were incubated directly when broth constituted the heating substrate. The capillary tubes (after being treated with phenol and washed with sterile water) were inserted into culture tubes. If, after 24-hour incubation, no growth was observed, the outside of the capillary was assumed to be sterile. Then, in order to liberate the heated bacteria the capillary tube was broken up with a sterile glass rod. Representative tubes, showing growth after the heating period, were examined in order to make certain that the growth was not due to contaminants.

RESULTS AND DISCUSSION

Preliminary studies were made with four strains of micrococci. When it became apparent that the same general trend was exhibited by all, one strain—a *Streptococcus*, No. 7A—was selected and studied extensively. A comparison is shown of the heat-resistance values obtained when different

concentrations of this organism were heated under the conditions indicated (Table 1).

The results indicate that moist bacteria when heated under moist conditions possessed a very low resistance to heat and were killed in 30 to 45 minutes at 55°C. Dry bacteria when heated under moist conditions were slightly more resistant and were killed in 45 to 60 minutes at 55°C. A greater resistance to heat was evident when these dry bacteria were heated in moist fat. In this substrate they were killed in 15 to 20 minutes at 100°C.(212°F.). A marked increase in resistance occurred, however, when they were heated in dry fat, since they then survived 135 minutes at 100°C. Finally, it was observed that the greatest resistance was obtained when the dry bacteria were heated in air. It is shown also that an increase in the number of bacteria very definitely influenced their final resistance.

TABLE 1

Comparison of Heat Resistance of a Micrococcus, No. 7A, Under Different Conditions in Thermal Death Time Tubes

Number of bacteria per tube	Condition of bacteria	Nature and condition of heating substrate	Temperature of heating	Resistance		Equivalent resistance at 100°C. (calculated z = 18)
				Survived	Killed	
			°C.	*min.*	*min.*	*min.*
400,000	Moist	Moist (broth)	55	30	45[1]	0.001
500,000	Dry	Moist (broth)	55	45	60	0.002
11,000,000	Dry	Moist (broth)	55	140	160	.....
500,000,000	Dry	Moist (broth)	55	290	305[1]	0.008
11,000,000	Dry	Moist (fat)	100	15	20	20
200,000,000	Dry	Moist (fat)	100	20	25	25
500,000	Dry	Dry (fat)	100	30	50[1]	50
11,000,000	Dry	Dry (fat)	100	135	150	150
5,000,000	Dry	Dry (fat)	115	40	50	1500
500,000	Dry	Air	110	30	65[1]	600
11,000,000	Dry	Air	110	135	150	1500
500,000,000	Dry	Air	110	220	230	2300
Resistance of 10-100 thousand moist spores of a putrefactive anaerobe, NCA No. 3679, under moist conditions in phosphate pH 7.....			115	25	30	900
Resistance of 25-30 million moist spores of a flat-sour organism, NCA No. 1503, under moist conditions in phosphate pH 7.....			115	30	35	1100

[1] Maximum and minimum values from two to three separate runs.

If the resistances obtained at the different temperatures are calculated according to the method of Ball (1928) to 100°C., using a slope of 18, the results are more easily compared as shown in the last column (Table 1). It is seen that the resistance of the micrococci varied from 0.001 minute to 2,300 minutes, depending on the conditions of the test.

It is noted also that the resistance of the micrococci under certain conditions exceeded the moist resistance of a sporeforming, putrefactive anaerobe and that of a highly resistant, flat-sour organism.

The results obtained when 500 million dried micrococci of Strain No. 7A were heated in air in various-sized tubes at 110°C.(230°F.) (Table 2) indi-

cate that the air apparently had no appreciable insulating effect, since the resistance was the same whether the sealed capillary tube was immersed in the heating bath directly or inclosed within the original thermal death time tube. In either case, the bacteria were killed in 10 to 15 minutes at 110°C. Marked differences in the heat resistance were observed, however, when the bacteria were heated in thermal death time tubes and in capillary tubes. In the capillary tubes the bacteria survived 10 minutes but not 15

TABLE 2

Comparison of Heat Resistance of a Dried Micrococcus, No. 7A, Under Dry Conditions in Various-Sized Tubes[1] at 110°C.(230°F.)

Kind of tube	Volume of tube	Resistance	
		Survived	Killed
	ml.	*min.*	*min.*
Capillary	0.04	10	15
Capillary in T.D.T.[2]	0.04	10	15
Capillary with bulb	1.96	25	35
Capillary with bulb, evacuated	1.96	150	160
Open capillary in T.D.T	3.85 (approx.)	150	160
T.D.T	3.85	220	230

[1] Five hundred million per tube. [2] T.D.T.—thermal death time tube.

minutes, whereas in the thermal death time tubes they survived 220 but not 230 minutes. When the volume of the capillary tube was increased by constructing a capillary with a bulb the resistance increased to 35 minutes. If such a tube was evacuated, the resistance increased to 160 minutes. If the capillary tube was left open and inserted in the thermal time tube, the resistance was increased to a killing time of 160 minutes.

The reason for the difference in heat resistance of dried bacteria heated under dry conditions in capillary tubes and in thermal death time tubes is not clear. It is evident, however, that when the bacteria were heated in tubes of restricted volume there was a destructive effect not accounted for

alone by the temperature to which they were exposed. The fact that evacuation of the tubes provided with bulbs resulted in a substantially greater resistance to heat leads to the hypothesis that during heating moisture, or some other constituent that is volatile at the temperature of exposure, is driven from the bacteria. This volatile matter, when under pressure as a result of the high temperature, may supplement the destructive effect of the heat.

SUMMARY AND CONCLUSION

Tests have been conducted which show that moist or dry micrococci heated under moist conditions are low in resistance. When heated in moist fat this resistance is higher. The resistance of dry micrococci in dry fat is exceptionally high and approaches that obtaining under conditions of dry sterilization.

From these experiments, it is possible to postulate that under certain conditions micrococci may survive the destruction of resistant spoilage organisms heated under usual conditions. The mechanism of fat protection in foods appears to rest upon the localized absence of moisture; subsequent failure of growth in the food is logically due to entrapment.

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EFFECTS OF METHOD OF SULFURING, DEHYDRATION, AND TEMPERATURE OF STORAGE ON ASCORBIC ACID AND CAROTENE CONTENT OF DEHYDRATED PEACHES[1]

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Dried fruits have long been a popular food and their post-war future is well assured. At present, according to Von Loesecke (1943), approximately 90 per cent of the freestone peaches are sun-dried and only about 10 per cent are artificially dried. Almost all of the sun-dried fruit is produced in California. Mrak (1942) stated that dehydrated peaches would not be acceptable to the trade because they acquire a dull, lemon-yellow color not found in the sun-dried fruit. Except for this one factor, however, Mrak considers dehydration superior to sun-drying. Certainly there are many regions where favorable weather conditions cannot be depended upon to make sun-drying practical. For that reason, it becomes important to develop dehydration methods for fruits which give acceptable products and preserve nutritive values.

The importance of sulfuring fruit prior to drying or dehydration has been definitely recognized and a complete discussion of its use is given by Mrak (1941). Sulfuring preserves ascorbic acid and carotene but destroys the thiamin. The retention of ascorbic acid and carotene during storage of dehydrated fruit depends on the retention of SO_2. The loss of SO_2 during storage is much more rapid if the moisture content of the fruit is high, 18 to 20 per cent. The optimum moisture content of dehydrated fruits is 12 to 14 per cent. The method of sulfuring which has been commonly used is exposure of the prepared fruit to SO_2 fumes in a closed box. The use of a solution of Na_2SO_3 for dipping the fruit has been recommended because it can be done more easily and the process can be carried on indoors. However, no report was found of the effect of this method of sulfuring on the nutritive value of the dried fruit.

Recent work by Cruess (1942) has shown that peaches of fairly good color can be secured without sulfuring if the fruit is steamed until cooked through before it is sun-dried. Dehydration of this fruit produced a lighter colored product but one less likely to spoil.

The most complete study of the effect of drying on the nutritive value of peaches was reported by Morgan and Field (1929). Vitamin C was determined in this study by the biological method. The results showed that sulfured peaches retained all their vitamin C whether they were sun-dried or dehydrated, while unsulfured peaches lost all their vitamin C content during drying. The Muir variety was used and the peaches were dehydrated for 20 to 24 hours at 63°C.(145.4°F.). The dehydrated, sul-

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fured peaches contained one biological unit per gram which is equivalent to 0.5 mg. of ascorbic acid.

The present study was undertaken to determine the effect of method of sulfuring, dehydration, and temperature of storage on the ascorbic acid and carotene content of peaches.

EXPERIMENTAL PROCEDURE

Statistical Design of Experiment:[2] A factorial design of experiment was used for the study. An amount of U. S. No. 1 Elberta peaches sufficient for the entire experiment was obtained from a local commercial orchard. The peaches were divided into six lots to be used for that number of replications. Replicates 1 and 2 were dehydrated two days after harvest; Replicates 3 and 4, three days; and 5 and 6, four days after harvest. The peaches were stored in a refrigerator until used in the experiment. They were peeled, halved, and pitted with stainless-steel knives. The halves were then thoroughly mixed and 10 samples were taken for each replicate. Two of these samples were sulfured by each of five methods and then dehydrated. After dehydration, the samples sulfured by the five methods were stored by two methods. Thus, 10 samples were studied in each replicate and a total of 60 samples was used for the experiment.

All data were analyzed by the analysis of variance. If the value for F was significant at the 5.0, 1.0, or 0.5 per cent level, the difference among means necessary for significance was calculated at the 5.0 per cent level.

Sulfuring Methods: Two sulfuring methods were used: SO_2 fumes and Na_2SO_3 solution. These methods were combined with a short steaming period which had been recommended by the California station because it was thought that it improved the retention of SO_2, thereby increasing the retention of carotene and ascorbic acid in the dried fruit, Jardine *et al.* (1944). The five sulfuring methods used for each 1,100-gram lot of peaches were (1) dipped in 1.5 per cent Na_2SO_3 solution for 15 minutes; (2) sulfured in SO_2 fumes in a sulfuring cabinet for 30 minutes; (3) blanched for five minutes in a rack suspended in steam at the temperature of boiling water and then poured from the rack into 1.5 per cent Na_2SO_3 solution and let stand for 15 minutes; (4) blanched in steam for five minutes, spread on trays and sulfured in SO_2 fumes for 30 minutes; and (5) unblanched to be used as check lots.

Dehydration Process: The 20 samples from the two replicates were randomized for each of the three drying periods. The dehydrator, Eheart and Sholes (1945a,b), was maintained at a temperature of 60 to 65.5°C. (140 to 150°F.). The peaches were dried until they became pliable and leathery which required 22.9 hours for the first drying period, 21.8 hours for the second, and 22.7 hours for the third period. The peaches were ripened three days in an airtight tin can in order to bring about equal distribution of moisture within each sample.

Storage Methods: After ripening, portions of the dehydrated samples were stored six months by each of two methods: (1) in fruit jars in a

[2] The statistical design of experiment was planned by Dr. Boyd Harshbarger, Statistician, Virginia Agricultural Experiment Station, and the analysis of the data was carried out under his supervision.

refrigerator at an average temperature of 5.6°C.(42.1°F.) and an average humidity of 59 per cent; (2) in fruit jars at an average room temperature of 23.9°C.(75.1°F.) and an average humidity of 42.8 per cent. The jars had three-piece lids with glass tops and rubber rings which fit the top of the jar. Tissue paper was used to completely fill the jar, thus decreasing the amount of air left around the peaches.

Analytical Methods: Ascorbic acid was determined in peaches after sulfuring, after dehydration, and after storage for six months of the dehydrated samples. Carotene analyses were made on the peaches after dehydration and after six months' storage of the dehydrated samples. The peaches were not analyzed for carotene after sulfuring since carotene determinations are very time-consuming and since the peaches are not ordinarily used at this stage. In order to determine the percentage destruction of carotene owing to sulfuring plus dehydration, however, 12 samples of raw (unsulfured) peaches were analyzed for carotene. Moisture determinations were made on the dehydrated samples so that the vitamin content could be reported on the dry basis.

The Morell (1941) method was used for all ascorbic acid determinations. Acetone was used, as described by Mapson (1941), to prevent the interference of SO_2 with the reduction of the dye.

The method used for carotene determinations was reported by Moore and Ely (1941), modified by Capps [3] and further modified by the authors (1945a,b).

For moisture determinations the dehydrated peaches were ground through a food chopper and samples were dried to constant weight in a vacuum oven at a temperature of 70°C.(158°F.).

RESULTS AND DISCUSSION

Data for retention of dry matter during sulfuring and the moisture content of dehydrated and stored (dehydrated) peaches are given (Table 1). The fresh peaches which had been stored four days before dehydration retained significantly more dry matter during sulfuring than those stored for two and for three days. It is not thought that the storage time was responsible for this difference but that it was due to different samples. As was expected, retention of dry matter varied according to method of blanching. The peaches which had been sulfured with SO_2 fumes retained the most dry matter, 96.52 per cent, which was a small significant loss probably owing to loss of peach juice on the trays. The peaches which had been steam blanched for five minutes then sulfured 15 minutes in a 1.5 per cent solution of Na_2SO_3 retained less dry matter than any of the others, 85.24 per cent. Those dipped in Na_2SO_3 for 15 minutes or steamed five minutes then exposed to SO_2 fumes 30 minutes did not differ significantly from each other and retained, respectively, 91.13 and 93.04 per cent of their dry matter.

The dehydrated peaches were low in moisture since they had an average content of only 10.46 per cent. After storage for six months the

[3] Personal communication from Dr. Julius Capps, Associate Animal Nutritionist, Alabama Agricultural Experiment Station, Auburn, Alabama.

moisture content was 10.59 per cent. The difference in moisture content owing to storage was not significant. Barger (1941) stated that the loss or gain of moisture by dried fruits was determined by the moisture deficit in fruit or air in relation to each other. Peaches of 20 to 25 per cent moisture content remained constant in weight at 75 per cent relative humidity. Raisins of 14 to 16 per cent moisture content changed slightly in weight at a relative humidity of 50 to 60 per cent. Evidently, in the present experiment, the jars were sufficiently sealed to prevent passage of moisture between the fruit and surrounding atmosphere.

TABLE 1

Effect of Storage Time After Harvest and Method of Sulfuring Peaches on Their Retention of Dry Matter During Sulfuring and on Their Moisture Content After Dehydration

Factors studied	Retention of dry matter	Moisture content of dehydrated samples	Moisture content of stored (dehydrated) samples
Storage time after harvest:[1]	*pct.*	*pct.*	*pct.*
2 days	91.45	10.97	11.46
3 days	89.19	10.27	10.73
4 days	93.81	10.13	9.58
Difference necessary for significance at 5% level	2.35	0.35	0.35
Method of sulfuring:[2]			
Na_2SO_3	91.13	10.57	10.80
SO_2	96.52	10.99	11.06
Steam—Na_2SO_3	85.24	10.06	10.13
Steam—SO_2	93.04	10.31	10.41
Unsulfured	.....	10.37	10.53
Difference necessary for significance at 5% level	2.71	0.32	0.45
Method of storing dehydrated samples:[3]			
Fruit jars—refrigerator	.....	.....	10.72
Fruit jars—room	.....	.....	10.45
Difference necessary for significance at 5% level	.....	.....	.....[4]
Means	.....	10.46	10.59

[1] All values given are means of 20 samples. [2] All values given are means of 12 samples. [3] All values given are means of 30 samples. [4] No significant difference between means.

The storage time after harvest affected the moisture content of dehydrated peaches. After dehydration, peaches which had been stored only two days after harvest contained more moisture, 10.97 per cent, than those stored three and four days, which contained 10.27 and 10.13 per cent moisture, respectively. There was no apparent cause for this difference owing to storage time after harvest. After storage of the dehydrated samples for six months there was a definite and significant decrease in moisture content of the peaches that had been stored for two, three, and four days after harvest. Those stored for two days contained 11.46 per cent moisture, for three days, 10.73 per cent, and for four days, 9.58 per cent. The reason for this relationship is not known at present.

The method of sulfuring affected the moisture content of dehydrated peaches since those which had been sulfured with SO_2 fumes contained more moisture, 10.99 per cent, than those sulfured by any of the other methods which gave variations from 10.06 to 10.57 per cent. After storage, the peaches sulfured with SO_2 fumes still contained more moisture, 11.06 per cent, than the others. The peaches which had been steamed and dipped in Na_2SO_3, those steamed and exposed to SO_2 fumes, and the unsulfured samples tended to be lowest in moisture, and those dipped in Na_2SO_3 were intermediate in moisture content.

The temperature of storage of the dehydrated peaches did not have a significant effect on moisture content since those stored in the refrigerator contained 10.72 per cent moisture and those at room temperature, 10.45 per cent.

TABLE 2

Effect of Method of Sulfuring and Method of Storing Dehydrated Samples on Ascorbic Acid Content of Sulfured, Dehydrated, and Stored (Dehydrated) Peaches [1]

Factors studied	Sulfured samples	Dehydrated samples	Stored (dehydrated) samples	
			Refrigerator	Room
Method of sulfuring:[2]	*mg./gm.*	*mg./gm.*	*mg./gm.*	*mg./gm.*
Na_2SO_3	.247	.061	.090	.013
SO_2	.245	.116	.162	.025
Steam—Na_2SO_3	.176	.067	.090	.015
Steam—SO_2	.157	.107	.133	.032
Unsulfured	.192	.025	.045	.040
Difference necessary for significance at 5% level	.040	.015	.035	.016
Method of storing dehydrated samples:[3]				
Fruit jars—refrigerator	.....	.....	.104	
Fruit jars—room	.....	.....	.025	
Difference necessary for significance at 5% level	.....	.....	.062	
Means	.206[4]	.075	.065	

[1] Figures are on dry basis. [2] All values given are means of 12 samples. [3] All values given are means of 30 samples. [4] Excluding unsulfured samples.

The ascorbic acid content of dehydrated peaches (Table 2) indicates that, when analyzed immediately after sulfuring, peaches which had been sulfured in Na_2SO_3 solution or in SO_2 fumes contained significantly more ascorbic acid, 0.247 and 0.245 mg. per gram,[4] respectively, than the unsulfured or raw peaches which had only 0.192 mg. per gram. This increase in ascorbic acid content was thought to have been due to reduction of dehydroascorbic acid by SO_2. The samples which were steamed for five minutes before sulfuring were not significantly different from the raw peaches. They contained 0.157 and 0.176 mg. per gram of ascorbic acid. Evidently there was a loss of ascorbic acid during steaming so that the effect of any increase during sulfuring was lost.

After dehydration the method of sulfuring had an entirely different effect on ascorbic acid values. The unsulfured peaches were the lowest of

[4] All data, unless otherwise stated, are reported on the dry basis.

all, having only 0.025 mg. per gram of ascorbic acid. Sulfuring peaches with SO_2 fumes was significantly better than with Na_2SO_3 solution since those treated by the former method contained 0.116 mg. of ascorbic acid per gram while those sulfured by the latter contained only 0.061 mg. Steaming prior to sulfuring made no difference in ascorbic acid losses since the values for dehydrated peaches sulfured with SO_2 and with Na_2SO_3 after steam blanching were 0.107 and 0.067 mg., respectively, values which were not significantly different from those for samples sulfured without steam blanching.

During storage for six months at a refrigerator temperature of 5.6°C. (42.1°F.) the dehydrated peaches increased significantly in ascorbic acid content. It had been found previously, Eheart and Sholes (1946), that dehydrated cabbage behaved similarly during cold storage. The reason for the apparent synthesis of ascorbic acid during cold storage is not at present understood. When the dehydrated peaches were stored for the same length of time at a room temperature of 23.9°C.(75.1°F.) there was a large loss of ascorbic acid, a loss so great that this temperature of storage should never be recommended if the preservation of ascorbic acid during storage is a consideration. The average ascorbic acid content of dehydrated peaches which had been stored at a cold temperature was 0.104 mg. per gram while that of peaches stored at room temperature was only 0.025 mg. per gram.

After six months of cold storage at 5.6°C.(42.1°F) of dehydrated peaches, the effect of method of sulfuring on the ascorbic acid content was the same as immediately after dehydration. The samples which had been sulfured with SO_2 fumes were still higher than those sulfured in Na_2SO_3 solution, whether steam blanched prior to sulfuring or not. The values were, as has been mentioned, higher than after dehydration, ranging from 0.090 mg. per gram for peaches sulfured in Na_2SO_3 to 0.133 and 0.162 mg. for those sulfured in SO_2. The unsulfured peaches remained the lowest in ascorbic acid content, having only 0.045 mg. per gram.

The average content of ascorbic acid for the raw samples was 0.192 mg. per gram and that for the sulfured samples was 0.206 mg. This increase in ascorbic acid owing to sulfuring was 7.3 per cent of the original amount. After dehydration, the average ascorbic acid content was 0.075 mg. per gram, which was only 39.1 per cent of that in the raw peaches. This loss was highly significant. After cold storage at 5.6°C.(42.1°F.) for six months, the dehydrated peaches contained 0.104 mg. per gram which was 54.2 per cent of the original amount in the raw peaches. After storage at a room temperature of 23.9°C.(75.1°F.), for the same length of time, only 13 per cent (0.025 mg. per gram) of the original amount in the raw peaches remained.

An average serving of dehydrated peaches, equivalent to 100 grams of raw peach, would furnish 1.09 mg. of ascorbic acid, a rather small contribution to the daily requirement. The peaches used in the experiment, however, were low in ascorbic acid when fresh, 100 grams of raw peach containing only 2.80 mg. of ascorbic acid. This value is low compared with the range of ascorbic acid given by Van Blaricom (1942) for a number of varieties of peaches. This author reported that the ascorbic acid content varied from 0.6 to 26.0 mg. per 100 grams of peach but the usual range was from seven to 14 mg.

The changes in carotene content of peaches during dehydration and storage of the dehydrated products (Table 3) show that the length of time the fresh peaches were stored before dehydration had a significant effect on the carotene content of the dehydrated peaches. The carotene content increased significantly from 28.3 μg. per gram for peaches stored two days to 32.1 μg. for those stored three days and 34.3 μg. for those stored four days. After six months of storage there were no longer differences in the carotene content of dehydrated peaches from fresh peaches which had been stored two, three, and four days before dehydration. The carotene content after storage ranged from 26.1 to 28.1 μg. per gram.

TABLE 3

Effect of Storage Time After Harvest, Method of Sulfuring, and Method of Storing Dehydrated Samples on Carotene Content of Dehydrated and Stored (Dehydrated) Peaches[1]

Factors studied	Dehydrated samples	Stored (dehydrated) samples
Storage time after harvest:[2]	μg./gm.	μg./gm.
2 days	28.3	27.7
3 days	32.1	26.1
4 days	34.3	28.1
Difference necessary for significance at 5% level	2.1	.....[5]
Method of sulfuring:[3]		
Na_2SO_3	31.9	27.3
SO_2	29.9	28.3
Steam—Na_2SO_3	32.1	29.3
Steam—SO_2	31.7	27.8
Unsulfured	32.3	23.7
Difference necessary for significance at 5% level	.....[5]	2.5
Method of storing dehydrated samples:[4]		
Fruit jars—refrigerator	.....	29.6
Fruit jars—room	.....	25.0
Difference necessary for significance at 5% level	.....	1.6
Means	31.6	27.3

[1] Figures are on dry basis. [2] These values are means of 20 samples. [3] These values are means of 12 samples. [4] These values are means of 30 samples. [5] No significant difference among means.

The method of sulfuring had no effect on the carotene content of peaches after dehydration (Table 3) and the values varied only from 29.9 to 32.3 μg. per gram. After storage for six months the samples sulfured by the four methods, i.e., with Na_2SO_3 solution, with SO_2 fumes, with steam plus Na_2SO_3 solution, and with steam plus SO_2 fumes, did not differ in their carotene content but the unsulfured samples were significantly lower. The carotene values for the sulfured lots ranged from 27.3 to 29.3 μg. per gram while the unsulfured peaches contained only 23.7 μg. per gram.

The temperature at which the dehydrated peaches were stored for six months significantly affected their retention of carotene. Those stored in a refrigerator contained 29.6 μg. per gram of carotene after storage while those stored at room temperature contained 25.0 μg. The effect of temperature of storage on the carotene content of peaches was similar to that

found for sweet potatoes, Eheart and Sholes (1945b). Thus, it would seem that untreated, dehydrated fruits and vegetables lose carotene more rapidly during storage than those which have been sulfured or blanched and that the rate of loss is accelerated by increased storage temperature.

The total losses of carotene from the fresh fruit were large. The mean carotene content of 12 samples of fresh peaches was 74.7 μg. per gram. After dehydration the mean value for carotene for all samples of peaches was 31.6 μg. per gram and after six months of storage, 27.3 μg. Thus, the peaches retained only 42.3 per cent of their original carotene content during dehydration and 36.5 per cent after six months of storage. Both the losses owing to dehydration and to storage were significant. Such a large loss of carotene was not expected since carotene is considered fairly stable to heat. However, Mitchell, Van Blaricom, and Roderick (1945) recently reported an average loss of 52 per cent of the carotenoids during the canning of a number of varieties of peaches.

An average serving of dehydrated peaches, equivalent to 100 grams of the raw peaches used in this experiment, would contain 461 μg. of carotene. This would be 15 per cent of the daily requirement of an adult man or woman for this vitamin, a substantial amount of the day's need. Van Blaricom (1942) reported a range of carotene from 1,000 to 2,000 I.U. for 100 grams of fresh peaches of a number of yellow varieties. This is equivalent to 600 to 1,200 μg., values somewhat higher than those found in the present work for dehydrated peaches.

SUMMARY AND CONCLUSIONS

Peaches were sulfured by four methods: dipping in a Na_2SO_3 solution, exposing to SO_2 fumes, and by each of these methods preceded by a five-minute steam blanch.

The peaches sulfured with SO_2 fumes retained the most dry matter and those steamed and dipped in Na_2SO_3 solution retained the least.

Peaches which had been sulfured with SO_2 fumes, whether steam blanched or not, retained more ascorbic acid during dehydration and after six months' storage of the dehydrated samples than those dipped in Na_2SO_3 solution. Unsulfured peaches were significantly lower than any of the sulfured samples after dehydration and after storage.

The dehydrated peaches stored at a refrigerator temperature of 5.6°C. (42.1°F.) showed significant increases in ascorbic acid while those stored at room temperature lost most of their ascorbic acid. Cold storage of dehydrated peaches should definitely be recommended for preservation of ascorbic acid.

The method of sulfuring made no difference in carotene retention during dehydration or after six months' storage of the dehydrated samples. The unsulfured samples, however, lost significantly more carotene during storage than those which had been sulfured.

A significant amount of carotene was lost from dehydrated peaches during storage for six months, and those stored at room temperature lost significantly more than those stored in the refrigerator.

An average serving of dehydrated peaches (equivalent to 100 grams of fresh peaches) would provide only 1.09 mg. of ascorbic acid but 461 μg. of carotene.

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DEFROSTING FROZEN FOODS BY HIGH-FREQUENCY HEAT

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For many years the defrosting of frozen foods has been a problem to the frozen-food user, especially the commercial user of the 30-pound carton or the 55-gallon barrel. At present, the defrosting of frozen foods is being poorly accomplished and the resulting thawed fruit is at times beyond use owing to discoloration and loss of flavor.

It has been found that high-frequency heat can be used successfully to defrost various fruits, vegetables, fish, and eggs in the package within minutes instead of hours or days.

EXPERIMENTAL PROCEDURE

All experiments were conducted with the use of a three-kilowatt, high-frequency unit manufactured by the Federal Telephone and Radio Corporation. This apparatus had a rated input of 4.6 kilowatts and a rated output of three kilowatts. The unit was operated on a frequency between 14 and 17 megacycles.

Frozen Eggs: Three types of frozen eggs were used for defrosting: egg yolks, egg whites, and whole eggs. The eggs were removed from the original 30-pound containers because the metal and circular construction of the containers prevented the maximum operation of the high-frequency unit. Representative samples were taken from each type of egg and repacked in Pyrex rectangular containers. In addition, one-pound samples were repacked in rectangular, closed paper boxes. The original and final temperatures were obtained by inserting a thermometer into the center of the sample being tested. An ice pick was used to bore a hole large enough for the insertion of the thermometer into the frozen sample.

Frozen Fruit: Thirty-pound cartons of frozen fruit, namely peaches and apricots, were subjected to high-frequency heat in the original, unopened, rectangular cardboard containers. Rectangular, retail, frozen-fruit packages, packed in paperboard, were also used. The original and final temperatures of the 30-pound packages were obtained by inserting the thermocouple of a Brown indicating pyrometer into the center of each. In the retail packages a thermometer was used to obtain the temperature readings.

Frozen Vegetables: In order to obtain a wide selection of vegetables, rectangular, retail frozen packages were used. Each package was subjected to high-frequency heat. A thermometer was used to obtain the original and final temperature readings.

Frozen Fish: Three types of frozen fish were used, namely haddock, red perch, and cod. These were packed in one-pound units and wrapped in cellophane. The unopened package was subjected to high-frequency heat after placing in a Pyrex dish and a thermometer was used to obtain the

original and final temperature. An ice pick was used to bore a hole into the center of the frozen fish so that the thermometer could be inserted. A thin film of water was added to the Pyrex dish in order to eliminate short circuiting.

RESULTS AND DISCUSSION

The experiments on defrosting frozen eggs showed that the egg yolks will defrost faster than the whole eggs and egg whites. In addition, the whole eggs will defrost faster than the egg whites. Although insignificant commercially, a thin film of egg which was adjacent to the container appeared to be slightly cooked. This was due to the cardboard heating faster than the eggs. From the experiments conducted with frozen eggs (Table 1), a commercial pack of 30 one-pound cartons can be defrosted within the original container provided eggs are packaged in a rectangular cardboard container.

TABLE 1

Defrosting Frozen Eggs

Type of egg	Weight of sample	Original temp.	Final temp. in high-freq. field	Time	Actual power output of 3-kw. unit	Air space
Pyrex dish	*lb.*	°*F.*	°*F.*	*min.*	*kw.*	*in.*
Egg yolks	5.0	10	52	10	1.9	1⅝
Whole eggs	3.5	10	42	10	1.7	1⅝
Egg whites	5.0	10	41	10	1.8	1⅝
Packaged						
Egg yolks	1.0	8	47	3	1.6	2¼
Whole eggs	1.0	7	42	3	1.7	2¼
Egg whites	1.0	7	42	3	1.9	2¼

The commercial, 30-pound containers of frozen apricots and peaches were successfully defrosted within the original container (unopened) in 15 minutes to a consistency so that they could be handled in the food plant. The retail frozen-fruit packages were defrosted to a temperature where they could be served as a dessert in the home; results are given (Table 2).

TABLE 2

Defrosting Frozen Fruit

Type of fruit	Weight of sample	Original temp.	Final temp. in high-freq. field	Time	Actual power output of 3-kw. unit	Air space
	lb.	°*F.*	°*F.*	*min.*	*kw.*	*in.*
Peaches	30	15	53	15	2.0	2.5
Apricots	30	17	50	15	1.7	3.0
	oz.					
Apple sauce	16	0	46	1	1.8	2.0
Blueberries	11	1	48	1	1.8	2.0
Cherries, R.S.P.	16	0	42	1	1.7	2.0
Plums	16	2	45	1	1.8	2.0
Peaches	16	2	43	1	1.6	2.0

Retail packages of frozen vegetables were successfully defrosted as is shown (Table 3) in two minutes. As in the experiments with frozen eggs and fruit, the vegetables did not change in color or flavor after thawing.

TABLE 3

Defrosting Frozen Vegetables

Type of vegetable	Weight of sample	Original temp.	Final temp. in high-freq. field	Time	Actual power output of 3-kw. unit	Air space
	oz.	*°F.*	*°F.*	*min.*	*kw.*	*in.*
Cut green beans	10	5	45	2	1.8	2.0
French green beans	10	5	45	2	1.8	2.0
Wax beans	10	6	47	2	1.8	2.0
Lima beans	12	5	45	2	1.8	2.0
Corn, whole kernel	10	5	48	2	1.8	2.0
Pea purée	12	5	46	2	1.7	2.0
Pumpkin pie mix	16	0	42	2	1.8	2.0
Pumpkin, cooked	16	0	44	2	1.8	2.0
Spinach	....	0	47	2	1.8	2.0
Squash, cooked	16	0	48	2	1.8	2.5
Succotash	11	0	47	2	1.8	2.0

Frozen fish was defrosted by high-frequency heat (Table 4) in 10 minutes to a temperature where it could be further processed, such as baked, broiled, or otherwise cooked. It was necessary to add a little water to the Pyrex dish so that the air gaps created between the dish and the wrapping material (cellophane) were eliminated.

TABLE 4

Defrosting Frozen Fish

Type of fish	Weight of sample	Original temp.	Final temp. in high-freq. field	Time	Actual power output of 3-kw. unit	Air space
	oz.	*°F.*	*°F.*	*min.*	*kw.*	*in.*
Cod fillets	16	2	47	10	1.8	2.5
Haddock fillets	16	2	49	10	2.2	2.5
Red perch fillets	16	2	50	10	2.0	2.5

The air space, which is the distance between the surface of the sample and the upper electrode, was found to cause considerable short circuiting when less than one inch was maintained. As the air space was increased, the short circuiting and burning were eliminated. The smaller the air space the more efficient is the output of the unit. There was less difficulty with burning when the frozen product was in syrup.

CONCLUSION

By using high-frequency heat the constant annoying problem of defrosting frozen foods can be solved. It is necessary to have the frozen food packed in rectangular cardboard containers. A maximum height of two and one-half to three inches is desirable, but not necessary. Frozen fruits,

eggs, and vegetables can be commercially defrosted in the plant as they are to be used. This can be accomplished without loss of quality, such as discoloration and loss of flavor.

In order to facilitate commercial defrosting, a conveyor could be set up so that the frozen food could be continuously passed through the high-frequency field. The speed of the conveyor could be correlated with the time desired to defrost the specific food in question.

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COMPOSITION OF SQUASHES AFTER WINTER STORAGE[1]

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Squashes were an important article of the diet and were extensively used in this locality for a considerable period before the arrival of the Colonists. According to Williams (1643), the name was given to this vegetable by the Indians; he says, "Askutasquash—their vine apples—which the English from them call squashes about the bignesse of apples of severall colours, a sweet, light wholesome refreshing." During the three hundred years that have elapsed since Roger Williams described the language and foods of the Indians of this locality, the horticulturists have modified greatly the squashes of the Indian days and developed numerous varieties of squashes, pumpkins, and gourds for food and ornamental purposes. For a large portion of the last three centuries both squashes and pumpkins have been extensively grown in this area as food for both man and animals. In fact the importance of this crop is so commonly appreciated that the usual manner of depicting autumn in New England is to picture a cornfield containing large shocks of corn generously interspersed with large golden pumpkins or squashes.

According to Bailey (1929), "The terms pumpkin and squash have little botanical significance and it is difficult to apply them arbitrarily.—the word pumpkin, and the colloquial punkin are derivatives from the old pompion, pumpion, pompeon which has its root in pepo or pepon—in North America the word pumpkin is habitually applied to the large-fruited forms of *Cucurbita pepo,* particularly to those varieties grown in field culture and that are sometimes used for stock-feeding, although it is given also to very large forms of *C. maxima.*"

In spite of the many years of extensive use of squashes for food, one can find very little data in the scientific literature concerning their nutritive value. Accordingly, the study reported here was undertaken to accumulate additional data concerning squashes, particularly their nutritive value at the end of the winter storage period.

EXPERIMENTAL PROCEDURE

Blue Hubbard is most extensively grown but it seemed desirable to study the composition of other varieties that are becoming commercially popular in this locality. Samples of four varieties, Blue Hubbard (*Cucurbita maxima* L), Butternut (*Cucurbita moschata* L), Golden Cushaw (*Cucurbita moschata* L), and Delicious (*Cucurbita maxima* L), were secured between February 8 and 18. Squashes were obtained from a chain grocery, from two independent grocery stores, from private homes, and from the storage house for the college dining hall. Thus a wide vari-

[1] Contribution No. 567 of the Massachusetts Agricultural Experiment Station.

ety of storage conditions was represented and the average of these conditions should be somewhat similar to those employed for squashes consumed in this locality. Presumably the squashes were harvested in October and they had been in storage for four months or more. Since the 22 samples were taken from individual squashes, the results of the assays supply data regarding individual variation in composition. Three of the Blue Hubbard squashes were rather small and apparently had been harvested before they were completely matured. Since the storage period for squashes was well advanced, only three Butternut and one Delicious squash could be found for assay.

Assay Procedures: Several portions were removed from various parts of the squash. The hard outer skin, the placenta, and the seeds were removed, and the remainder was promptly cut into very small pieces that were thoroughly mixed. The amount of carotene in the squashes was determined by the Wall and Kelley (1943) method for plant tissues. Ten grams of squash and 75 c.c. of solvent (30 parts acetone and 20 parts Skellysolve) were mixed in a Waring blendor for 10 minutes or until the squash was reduced to small particles and thoroughly mixed with the solvent. The mixture, which resembled a purée, was filtered through a sintered glass funnel with suction. The solid material was returned to the blendor and re-extracted a second and, if necessary, a third time or until the solid material was free of carotene. The extracts were combined, made up to 250-c.c. volume and filtered through five gm. anhydrous Na_2SO_4. A 50-c.c. aliquot was evaporated to approximately 10 c.c., eluted through a column of activated magnesium oxide and hyflo super-cel, and the carotene content was measured with a Coleman spectrophotometer. The entire carotene assay was carried out in a darkened room which had about three-foot candles of illumination.

The procedure used for determining the riboflavin content of the squashes was as follows: Twenty grams of finely minced squash and 50 c.c. of 0.25N H_2SO_4 were rapidly agitated in a Waring blendor for 10 minutes. The mixture was boiled for an hour under a reflux condenser and cooled. A sufficient quantity of a 6.5-per cent solution of Na_3PO_4 was added to adjust the pH to 6.5 and the mixture was diluted with water to 250 c.c. After the solid material had settled, a 20-c.c. aliquot was transferred to a 100-c.c. volumetric flask, 30 c.c. of water was added, $KMnO_4$ solution was added drop-wise until a pink color remained, sufficient hydrogen peroxide was added to discharge the color and acetone sufficient to make 100 c.c. The mixture was filtered, and 20-c.c. aliquots were treated and read in a Coleman Universal spectrophotometer, as described by Holmes, Jones, Wertz, and Kuzmeski (1943). The entire assay procedure for riboflavin was carried out in a darkened room with approximately three-foot candles of illumination, and amber glassware was used.

Recognized assay procedures were used to determine the water, calcium, iron, magnesium, and phosphorus contents of the squashes (i.e., the oxalate method for calcium, colorimetric for iron, magnesium pyrophosphate for magnesium, volumetric for phosphorus), all of which are official methods of the Association of Official Agricultural Chemists (1940).

DISCUSSION OF RESULTS

A careful analysis of the data obtained from the assays of the four varieties of squashes (Table 1) reveals that the most significant difference in the composition of the four varieties is the carotene content. While the Delicious appears to be much richer than the Blue Hubbard, the Butternut and Golden Cushaw had considerably more than three times as much carotene as the Blue Hubbard. The four varieties of squashes contained about the same amount of riboflavin. The Butternut squash had higher calcium, iron, magnesium, and phosphorus contents than the other varieties, but since only three squashes were assayed, these values may not indicate true varietal differences.

TABLE 1

Composition of Squashes[1] After Winter Storage

Sample No.	Variety	Water	Carotene	Riboflavin	Calcium	Iron	Magnesium	Phosphorus
		pct.	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*
1	Blue Hubbard[2]	90.30	1.90	.10	18	.51	20	63
2	Blue Hubbard	93.79	2.80	.10	17	.35	8	12
3	Blue Hubbard	85.42	3.50	.19	19	.81	13	43
4	Blue Hubbard	90.82	1.48	.16	19	.50	7	35
5	Blue Hubbard	92.70	0.74	.18	15	.49	13	27
6	Blue Hubbard	91.21	2.79	.17	16	.45	9	40
7	Blue Hubbard	91.36	2.30	.08	21	.85	6	32
8	Blue Hubbard	88.04	2.75	.09	29	.76	9	38
9	Blue Hubbard	90.69	1.90	.07	15	.65	14	42
10	Blue Hubbard	88.48	2.04	.17	18	.59	22	40
11	Blue Hubbard	92.09	3.00	.22	18	.40	6	15
12	Blue Hubbard	92.46	1.45	.12	19	.59	6	24
	Average	90.61	2.22	.14	19	.58	11	34
13	Butternut	86.47	7.68	.10	32	.87	19	71
14	Butternut	87.02	8.85	.13	76	.74	13	60
15	Butternut	87.50	7.30	.12	41	.69	14	72
	Average	87.00	7.94	.12	50	.77	15	68
16	Golden Cushaw	89.99	4.20	.13	16	.64	7	28
17	Golden Cushaw	88.56	7.50	.11	19	.87	7	29
18	Golden Cushaw	92.90	6.40	.13	14	.43	6	19
19	Golden Cushaw	88.50	5.80	.18	19	.59	8	30
20	Golden Cushaw	85.96	10.90	.18	14	.90	11	37
21	Golden Cushaw	90.51	7.90	.18	13	.65	10	30
	Average	89.40	7.12	.15	16	.68	8	29
22	Delicious	89.75	3.90	.14	15	.64	25	41

[1] Fresh basis. [2] Samples 1 to 9, inclusive, were taken from mature squashes; 10 to 12, inclusive, were taken from immature squashes.

It is interesting to compare the high carotene values, 7.94 mg. and 7.12 mg. per 100 gm., obtained for the Butternut and Golden Cushaw squashes, respectively, with values previously reported by Holmes, Spelman, and Jones (1945) for immature squashes of these varieties. The immature Butternut and Golden Cushaw squashes contained 3.31 mg. and 6.21 mg.

of carotene per 100 gm., respectively, which are much lower than the values found at the end of the winter storage period. This observation raises a question as to whether the carotene content of these squashes increases as the squashes mature or whether the carotene content increases during winter storage or both. Pyke and Charkey (1943) found that six varieties of carrots grown in Colorado contained two or three times as much carotene in the large mature stage as when they were small and young. Snyder and Lachman (1944) report that each of seven varieties of carrots increased in carotene content during the entire growing period and also increased during cold storage from November to April. On the other hand, Pepkowitz, Larson, Gardner, and Owens (1944) found that the carotene content of each of 10 varieties of carrots increased from the 76th day after planting until the 90th day following planting and then decreased. Accordingly, studies are in progress to accumulate additional data concerning factors that influence the variations in the carotene content of squashes.

The carotene content of the Butternut and Golden Cushaw squashes, 7.94 mg. and 7.12 mg. per 100 gm., respectively, is higher than that of almost all the fruits and vegetables included in the human diet. In their determination of pure carotene in green vegetables Fraps, Meinke, and Kemmerer (1941) found green string beans contained 0.03 mg.; beet tops, 3.2 mg.; sweet potatoes, 4.5-5.7 mg.; and carrots, 4.5-8.4 mg. of carotene per 100 gm. Zimmerman, Tressler, and Maynard (1941) reported from a study of carotene in fresh and frozen vegetables that bush lima beans contained 0.24 mg.; spinach, 0.46 mg.; asparagus, 0.70 mg.; and broccoli, 1.0 mg. per 100 gm. Pyke and Charkey (1943) reported that the average carotene content of six varieties of summer-grown carrots ranged from 14.6 to 25.5 mg. per 100 gm. and that similar fall-grown carrots ranged from 16.1 to 28.2 mg. per 100 gm.

Booher and Marsh (1941) assigned the following values to the raw fruits and vegetables they found richest in carotene: green snap beans, 0.7 mg.; beet greens, 9.7 mg.; chard, 8.7 mg.; collards, 5.3 mg.; endive, 2.3 mg.; kale, 6.3 mg.; peach (Elberta), 1.0 mg.; sweet potatoes, 2.3 mg.; tomatoes, 0.9 mg.; and turnip greens, 9.4 mg. of carotene per 100 gm. Sheets, Leonard, and Gieger (1941) studied the distribution of minerals and vitamins in different parts of leafy vegetables and found the carotene content of the leaf blades was as follows: chard, 6.9 mg.; beet tops, 7.6 mg.; spinach, 7.6 mg.; lettuce, 11.2 mg.; radish, 7.3 mg.; turnip tops, 10.1 mg.; rape, 5.9 mg.; and collards, 3.7 mg. per 100 gm. Fitzgerald and Fellers (1938) determined the carotene content of fresh market vegetables and reported the following range of value: tomatoes, 0.3-1.8 mg.; carrots, 1.0-5.0 mg.; sweet peppers, 0.5-4.6 mg.; pumpkin and squash, 1.1-12.0 mg.; spinach, 4.0-15.0 mg.; sweet potatoes, 0.6-4.2 mg.; and peaches (yellow), 0.2-1.8 mg. per 100 gm., for fruits and vegetables richest in carotene. The data cited above are for the fruits and vegetables that contain the largest recorded amounts of carotene. A large proportion of the fruits and vegetables grown in this country contain very small amounts of carotene and many have no detectable carotene. Thus, it is quite evident that very few of the many fruits and vegetables used for human food, supply as much carotene as the Butternut and Golden Cushaw under discussion. In fact,

the Blue Hubbard and Delicious squashes discussed above contained much more carotene than is found in a large proportion of the fruits and vegetables commonly included in the human dietary. Incidentally, it must be constantly remembered that the mere finding of relatively large amounts of carotene in plant tissues does not necessarily yield conclusive evidence regarding their real or relative value as a source of carotene for human nutrition because, obviously, the true nutritive value of any fruit or vegetable as a source of carotene depends wholly upon the amount of carotene that the human body can obtain from that food material.

The data regarding the amount of riboflavin, calcium, iron, magnesium, and phosphorus found in the 22 squashes examined at the end of the winter storage period (Table 1) show this vegetable to be a valuable source of these essential food constituents. However, the squashes contained less calcium, iron, and magnesium but considerably more riboflavin and phosphorus than Holmes and Crowley (1944) found in lettuce grown in this locality. Also Holmes, Crowley, and Kuzmeski (1945) reported that freshly picked kale grown in this area contained more calcium, iron, magnesium, and phosphorus than the squashes under consideration.

The values obtained for the carotene, riboflavin, calcium, iron, magnesium, and phosphorus content of the squashes at the end of the winter storage period varied with the different varieties but they also varied considerably with the individual squashes within the varieties. Since no data were available regarding the ancestry of the seed, soil, fertilizer, cultural and storage conditions, no conclusions can be drawn concerning the influence of these or other factors upon variation in composition of the different squashes.

SUMMARY

Samples of 22 squashes from four varieties of winter squashes, Blue Hubbard (*Cucurbita maxima* L), Butternut (*Cucurbita moschata* L), Golden Cushaw (*Cucurbita moschata* L), and Delicious (*Cucurbita maxima* L), were assayed at the end of a four months' winter storage period for water, carotene, riboflavin, calcium, iron, magnesium, and phosphorus. The respective average values obtained were for Blue Hubbard 90.61 per cent and 2.22, 0.14, 19, 0.58, 11, and 34 mg. per 100 gm.; for Butternut 87 per cent and 7.94, 0.12, 50, 0.77, 15, and 68 mg.; for Golden Cushaw 89.40 per cent and 7.12, 0.15, 16, 0.68, 8, and 29 mg.; and for Delicious 89.75 per cent and 3.90, 0.14, 15, 0.64, 25, and 41 mg. per 100 gm. The results obtained in this study show that Blue Hubbard, Butternut, Golden Cushaw, and Delicious squashes, after four months' winter storage, are good sources of food ingredients essential for the human diet. The most striking values obtained were for carotene, the Butternut and Golden Cushaw being unusually rich in this essential food constituent.

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RELATIONSHIP BETWEEN PHOSPHORUS FRACTIONS IN SOIL AND PROTEIN ACCUMULATION IN LEAVES OF CEREALS[1]

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The chemical characteristics of a group of soils in the vicinity of Midland, Douglas County, Kansas, have been described in detail by Wynd and Romig (1943). Wynd and Noggle (1943) have reported that the amounts of phosphorus in these soils present in the fractions designated as P_1, chemosorbed, and chemosorbed plus easily-acid-soluble were positively correlated with the amounts of organic matter and nitrogen present. These fractions were composed largely of chemosorbed phosphorus. They were also related to the amount of replaceable bases in many instances because the organic matter is responsible for the major part of the base-exchange capacity in many soils, and the base-exchange capacity often governs the amount of replaceable bases retained in the soil. The amounts of easily-acid-soluble phosphorus were not related to any of the soil properties dependent on the organic matter.

Wynd and Noggle (1946) reported that, in the soils studied, the amounts of phosphorus in the fractions P_1, chemosorbed, and chemosorbed plus easily-acid-soluble were directly proportional to the growth of oats and rye, but this was true only if the amounts of phosphorus were proportional to the amount of organic matter and nitrogen in the soil. In soils in which this association of properties did not exist, the amount of replaceable bases determined the yield of dry matter of these species. It thus becomes evident that the amount of replaceable bases, and not the phosphorus present in these fractions, was the factor limiting growth. The amounts of easily-acid-soluble phosphorus were shown to bear no relation to the growth of oats and rye.

Since phosphorus takes part in a variety of biochemical processes and is a component of some types of proteins, it is of interest to determine what relationships exist between various fractions of soil phosphorus and the amounts of protein in the leaves of cereals. The present study describes the relationships between the phosphorus fractions P_1, chemosorbed, chemosorbed plus easily-acid-soluble, and easily-acid-soluble present in the top soils of 15 fields in the vicinity of Midland and the percentages and yields of protein in the leaves of oats and rye.

MATERIALS AND METHODS

The details of the analytical procedure are described in the authors' previous paper (1943); the chemical data for the phosphorus fractions are presented (Table 1). All references in the text to additional chemical properties of these soils refer to data presented in the tables of Wynd and

[1] The expenses incurred during the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

Romig (1943). The data for the protein accumulation in the leaves of the crops are presented (Table 2). The M.S.C. file numbers refer to the original samples preserved in the Department of Botany, Michigan State College. Further chemical details of the soils studied may be obtained by referring to the tables of Wynd and Romig (1943) by means of corresponding file numbers. The point numbers enable the reader to identify in the tables the soils from which each of the data presented in the figures was obtained.

The crops were harvested just as jointing occurred and were quickly dried in an Arnold hot-air dehydrator. This stage of maturity of the plants was selected as the basis for comparison because the leaves contain especially high concentrations of protein and other nutritive substances at this stage of physiological development.

EXPERIMENTAL RESULTS

P_1 Phosphorus Fractions: The data presented (Figs. 1 and 2) show that the amounts of soil phosphorus in the P_1 fraction are usually positively related to the percentage of protein in the leaves of both oats and rye. Soils represented by Points 1, 2, 5, and 7 (Fig. 1) contain comparatively large amounts of nitrogen. Of this group of four soils, the one represented by Point 1 contains the least nitrogen and it also produced the lowest percentage of protein in the leaves of oats. The amount of nitrogen in the soil appears to be more important than the amount of phosphorus in the P_1 fraction in governing the percentage of protein in the leaves.

The total yields of protein in pounds per acre are shown (Figs. 3 and 4) to be roughly proportional to the magnitudes of the P_1 phosphorus fraction. Point 7 (Fig. 3) represents a soil which contains an exceptionally large amount of replaceable bases. The location of this point in the figures suggests that the total yield of protein is influenced more by the replaceable bases than by the P_1 phosphorus fraction in the soil.

Chemosorbed Phosphorus Fraction: In general, the soils which contain the greater amounts of chemosorbed phosphorus produce the higher concentration of protein in the leaves of oats and rye (Figs. 5 and 6).

The total yields of protein are shown (Figs. 7 and 8) to be rather closely related to the amount of chemosorbed phosphorus in the soil. Point 7 (Fig. 7) represents a very high yield of protein, even though this soil contains only a moderate amount of chemosorbed phosphorus. This soil contains conspicuously more replaceable bases than the other soils in the group, and apparently the replaceable bases are more important than chemosorbed phosphorus in determining the total yield of protein.

The soils represented by Points 6, 7, and 8 (Fig. 8) contain increasing amounts of nitrogen and replaceable bases in the order named, and these associated factors are also important influences in the yield of protein.

Chemosorbed Plus Easily-Acid-Soluble Phosphorus Fraction: The data presented (Figs. 9 and 10) show that the percentages of protein in the leaves of both oats and rye are closely related to the amount of phosphorus in the chemosorbed plus easily-acid-soluble fraction.

The total yields of protein are shown (Figs. 10 and 11) to be closely related to the amount of phosphorus present in the chemosorbed plus

TABLE 1

Phosphorus Fractions in Soils[1]

M.S.C. file No.	Field No.	Point No.	Phosphorus fractions			
			P_1	Chemo sorbed	Chemo-sorbed plus easily-acid-soluble	Easily-acid-soluble
			p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
267	2A	1	105	118	176	58
268	2B	2	104	90	168	78
269	19	3	58	60	110	50
270	11E	4	76	63	95	32
272	12	5	165	113	170	57
274	14B	6	67	60	138	78
280	18	7	100	93	160	67
286	9	1	60	38	107	69
285	10	2	56	45	128	83
288	20	3	70	50	112	62
284	19B	4	45	38	88	50
287	22	5	51	35	98	63
291	11	6	74	63	134	71
289	15A	7	100	78	170	92
290	15B	8	104	88	197	109

[1] The first group of points, 1 to 7 inclusive, refers to soils planted with oats; the second group of points, 1 to 8 inclusive, refers to soils planted with rye.

TABLE 2

Percentage and Yield of Protein in Cereal Leaves[1]

M.S.C. file No.	Field No.	Point No.	Date harvested	Yield of dry matter per acre	Protein (N×6.25)	Yield of protein per acre
				lb.	*pct.*	*lb.*
315	2A	1	Oct. 8, 1940	221	25.81	57.04
317	2B	2	Oct. 11, 1940	304	28.88	87.80
319	19	3	Oct. 17, 1940	200	18.80	37.60
320	11E	4	Oct. 18, 1940	200	22.00	44.00
321	12	5	Oct. 21, 1940	389	24.06	93.59
318	14B	6	Oct. 23, 1940	253	23.38	59.15
325	18	7	Nov. 1, 1940	552	27.94	154.23
332	9	1	Apr. 10, 1941	144	18.00	25.92
334	10	2	Apr. 11, 1941	221	16.75	37.02
335	20	3	Apr. 12, 1941	379	21.88	82.93
336	19B	4	Apr. 13, 1941	500	16.94	84.70
337	22	5	Apr. 14, 1941	298	16.94	48.81
338	11	6	Apr. 17, 1941	947	21.94	207.77
339	15A	7	Apr. 20, 1941	1430	23.56	336.91
340	15B	8	Apr. 20, 1941	1950	20.50	403.85

[1] The first group of points, 1 to 7 inclusive, refers to oats; the second group of points, 1 to 8 inclusive, refers to rye.

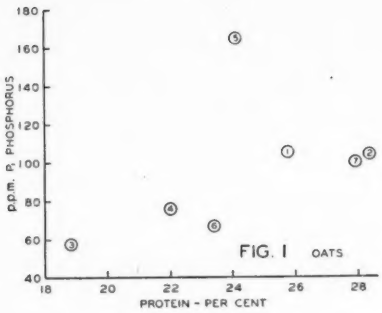

FIG. 1 OATS

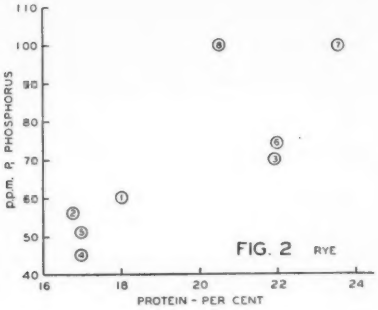

FIG. 2 RYE

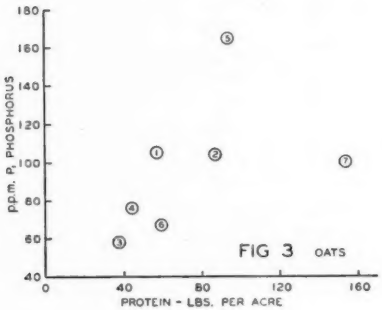

FIG 3 OATS

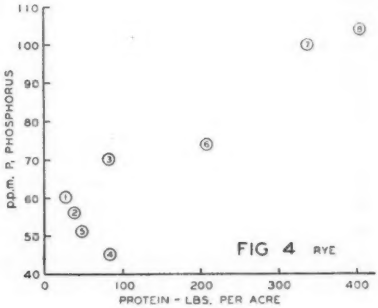

FIG 4 RYE

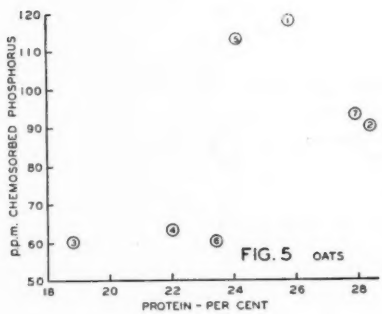

FIG. 5 OATS

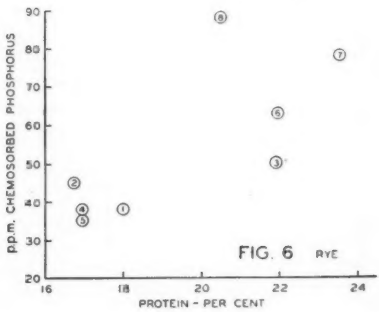

FIG. 6 RYE

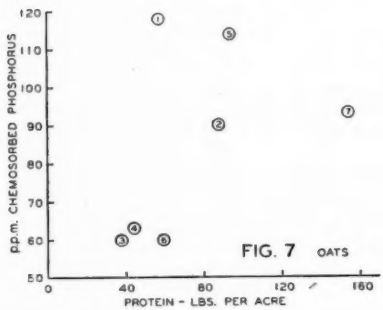

FIG. 7 OATS

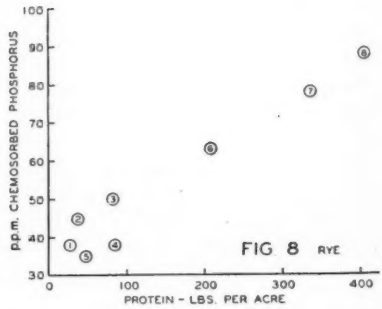

FIG. 8 RYE

easily-acid-soluble fraction. Point 1 (Fig. 11) represents a soil rich in nitrogen and phosphorus but exceptionally poor in replaceable bases. Its position in the figure shows that the amount of replaceable bases in the soil is more important than this phosphorus fraction in determining the total yield of protein in the crop.

Easily-Acid-Soluble Phosphorus: A vaguely defined positive relationship between the amounts of easily-acid-soluble phosphorus and the percentages of protein in the leaves of oats and rye is noticeable (Figs. 13 and 14), although the points in the figures are too scattered to establish this relationship with certainty.

In general, a positive relationship is shown (Figs. 15 and 16) between the amounts of easily-acid-soluble phosphorus in the soil and the total yields of protein in the leaves of oats and rye, although it is difficult to interpret the erratic distribution of the points. The data previously presented by Wynd and Romig (1943) show that the yields of protein described in the present paper are roughly proportional to the amounts of replaceable bases which the soils contain.

DISCUSSION

There appears to be a positive correlation between the percentages of protein in the leaves of oats and rye and the amounts of soil phosphorus in the fractions: P_1, chemosorbed, and chemosorbed plus easily-acid-soluble. However, a study of the exceptional instances indicated in the figures shows that these relationships exist only if the higher amounts of phosphorus accompany the higher amounts of nitrogen in the soil. If this association of the amounts of phosphorus and of nitrogen does not exist, then the amount of nitrogen determines the percentage of protein in the leaves of both oats and rye.

The association of the amounts of easily-acid-soluble phosphorus is shown (Figs. 13 and 14) to be poorly defined. This is probably due to the fact, reported by Wynd and Noggle (1946), that this fraction of soil phosphorus is not associated with any of the nutritional factors associated with the organic matter in the soil.

There also appears to be a positive correlation between the amounts of phosphorus present in the P_1, chemosorbed, and chemosorbed plus easily-acid-soluble fractions and the total yield of protein in the leaves of oats and rye. A study of the exceptional cases shows that the association between these fractions and the yields of protein exists only if the higher amounts of phosphorus are accompanied by higher amounts of replaceable bases.

The relationships of the amounts of easily-acid-soluble phosphorus in the soil to the yields of protein are difficult to interpret. There appears to be a vaguely positive relationship, but this is probably due to the amounts of bases in the soils rather than to the amount of phosphorus in this fraction.

The data presented show that the association of factors composing the nutritional complex in the soil leads the student of plant nutrition to false interpretation of data unless many different factors are measured simultaneously. The present study was based on a group of 15 soils in

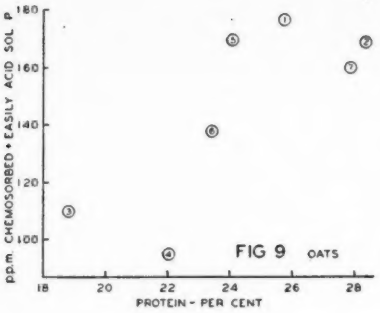

FIG 9 OATS

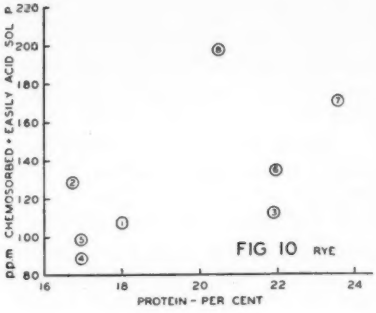

FIG 10 RYE

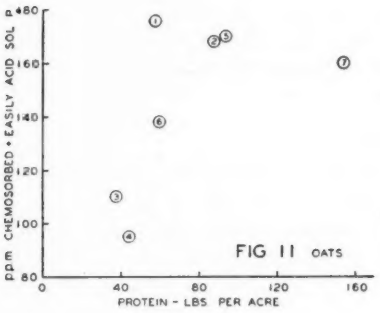

FIG 11 OATS

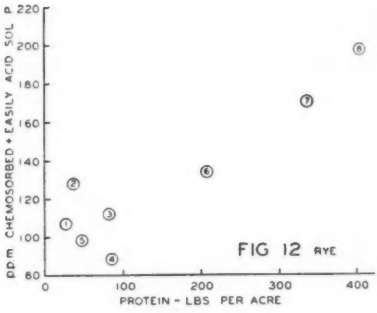

FIG 12 RYE

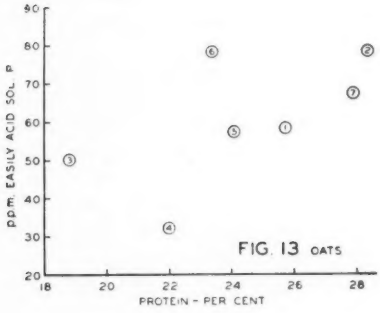

FIG. 13 OATS

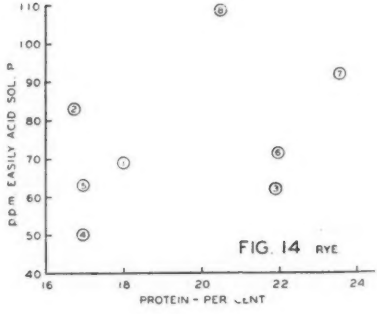

FIG. 14 RYE

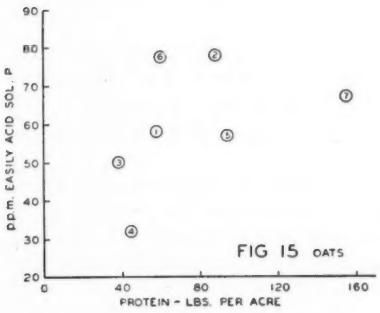

FIG 15 OATS

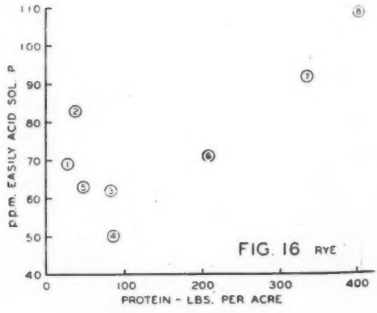

FIG. 16 RYE

the vicinity of Midland, Douglas County, Kansas, and generalizations must be avoided until a large mass of data has been accumulated from many different soils.

SUMMARY

The amounts of phosphorus present in each of four fractions designated as P_1, chemosorbed, chemosorbed plus easily-acid-soluble, and easily-acid-soluble were determined in 15 soils in the vicinity of Midland, Douglas County, Kansas. The percentages and total yields of protein in the leaves of oats and rye, grown on each of these soils and harvested at the jointing stage, were determined.

The percentage of protein in the leaves is related to the amount of phosphorus present in the surface soil in the P_1, chemosorbed, and chemosorbed plus easily-acid-soluble fractions but *only* if these amounts of phosphorus were positively correlated with the amounts of nitrogen in the soil.

The yield of protein in the leaves is also positively related to the amount of phosphorus present in the P_1, chemosorbed, and chemosorbed plus easily-acid-soluble fractions but *only* if these amounts of phosphorus are positively correlated with the amounts of replaceable bases in the soil. The amounts of replaceable bases dominated the effect of phosphorus in governing the total yield of protein in the leaves because of the effect the bases exert on growth.

The amounts of easily-acid-soluble phosphorus exhibited no clearly defined relationship to the percentage or to the yield of protein in the leaves.

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RELATIONSHIP BETWEEN PHOSPHORUS FRACTIONS IN SOIL AND CAROTENE IN LEAVES OF CEREALS[1]

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(Received for publication, July 23, 1945)

The chemical characteristics of a group of soils in the vicinity of Midland, Douglas County, Kansas, have been reported by Wynd and Romig (1943). The amounts of phosphorus in these soils present in various fractions were shown by Wynd and Noggle (1943) to be related to the amounts of nitrogen and organic matter, provided that the fraction was composed largely of chemosorbed phosphorus. The amounts of easily-acid-soluble phosphorus were not related to any of the chemical factors studied.

The relationship between the amounts of phosphorus present in these soils in various fractions and the growth of oats and rye, and upon the percentages and yields of protein in the leaves of these cereals have been reported by Wynd and Noggle (1946a,b).

The purpose of the present paper is to report the relationship between the amounts of soil phosphorus present in various fractions and the amounts of carotene in the leaves of oats and rye. Very little is known concerning the effects of nutritional conditions on the vitamin content of plants. The increasing use of immature cereals as sources of vitamins makes the study of the effects of soil properties on the vitamin content of plants of especial importance.

MATERIALS AND METHODS

Oats and rye were grown on the soils described by Wynd and Noggle (1943) and harvested just as jointing occurred, and the material was dried immediately in an Arnold hot-air dehydrator. The crops were harvested at this stage because the leaves contain high concentrations of vitamins during this period of their physiological development. The samples were taken from the same harvests that furnished the material used for the study of the protein content, Wynd and Noggle (1946b).

The analytical procedures for the determination of the various phosphorus fractions have been reported in detail by Wynd and Noggle (1943). The carotene was determined by the method of Buxton and Dombrow (1938) modified as described by Wynd and Noggle (1943).

The data for the phosphorus fractions appear (Table 1) and those for the carotene content of the leaves (Table 2). The M.S.C. file numbers refer to the original samples preserved in the Department of Botany, Michigan State College. Additional chemical data for the soils studied may be obtained by referring to the corresponding file numbers in the tables published by Wynd and Romig (1943), and all comments in the text concerning additional chemical properties of the soils included in the

[1] The expenses incurred by the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

TABLE 1

Phosphorus Fractions in Soils[1]

M.S.C. file No.	Field No.	Point No.	Phosphorus fractions			
			P_1	Chemosorbed	Chemosorbed plus easily-acid-soluble	Easily-acid-soluble
			p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
267	2A	1	105	118	176	58
268	2B	2	104	90	168	78
269	19	3	58	60	110	50
270	11E	4	76	63	95	32
272	12	5	165	113	170	57
274	14B	6	67	60	138	78
280	18	7	100	93	160	67
286	9	1	60	38	107	69
285	10	2	56	45	128	83
288	20	3	70	50	112	62
284	19B	4	45	38	88	50
287	22	5	51	35	98	63
291	11	6	74	63	134	71
289	15A	7	100	78	170	92
290	15B	8	104	88	197	109

[1] The first group of points, 1 to 7 inclusive, refers to soils planted with oats; the second group of points, 1 to 8 inclusive, refers to soils planted with rye.

TABLE 2

Concentration and Yield of Vitamin A (Carotene)[1]

M.S.C. file No.	Field No.	Point No.	Date harvested	Yield of dry matter per acre	Carotene	Carotene per acre
				lb.	*mg./100 gm.*	*lb.*
315	2A	1	Oct. 8, 1940	221	41.8	.092
317	2B	2	Oct. 11, 1940	304	49.8	.151
319	19	3	Oct. 17, 1940	200	33.3	.067
320	11E	4	Oct. 18, 1940	200	45.8	.178
321	12	5	Oct. 21, 1940	389	37.0	.094
318	14B	6	Oct. 23, 1940	.....	.....	.....
325	18	7	Nov. 1, 1940	552	44.3	.245
332	9	1	Apr. 10, 1941	144	32.0	.046
334	10	2	Apr. 11, 1941	221	29.0	.064
335	20	3	Apr. 12, 1941	379	33.0	.125
336	19B	4	Apr. 13, 1941	500	28.3	.142
337	22	5	Apr. 14, 1941	298	25.8	.0769
338	11	6	Apr. 17, 1941	947	32.0	.303
339	15A	7	Apr. 20, 1941	1430	37.8	.541
340	15B	8	Apr. 20, 1941	1950	32.3	.636

[1] The first group of points, 1 to 7 inclusive, refers to oats; the second group, 1 to 8 inclusive, refers to rye.

present study are based on the data in these tables. The point numbers enable the reader to identify in tables the individual soils which furnished each of the data presented in Table 2 and in the figures.

EXPERIMENTAL RESULTS

P_1 Phosphorus Fraction: The data presented (Figs. 1 and 2) show that a very definite relation exists between the amounts of phosphorus in the soil in the P_1 fraction and the concentration of carotene in the leaves of oats and rye. This relationship is especially evident for rye.

The total yield of carotene in pounds per acre is also related to the amount of phosphorus in the soil in the P_1 fraction. Point 7 (Fig. 3) represents a soil very rich in replacable bases. The large amount of growth which was produced by these bases evidently was more important than the amount of P_1 phosphorus in determining the total yield of carotene.

Chemosorbed Phosphorus Fraction: The concentration of carotene in the leaves is shown (Figs. 5 and 6) to be closely related to the amount of chemosorbed phosphorus in the soil. This relationship is especially evident for rye.

The total yield of carotene is also related to the chemosorbed phosphorus fraction (Figs. 7 and 8). Points 1 and 7 (Fig. 7) represent soils rich in nitrogen, and the concentration of carotene in the crop produced by them is high, yet the yields of carotene for these soils are very different. The soil represented by Point 1 contains a very low concentration of replaceable bases, while the soil represented by Point 7 contains a very high concentration of bases. The amount of replaceable bases, because of its effect on growth, is more important in governing the yield of carotene than is the amount of chemosorbed phosphorus in the soil.

Chemosorbed Plus Easily-Acid-Soluble Phosphorus Fraction: The concentrations of carotene in the leaves are shown (Figs. 9 and 10) to be related to the amount of phosphorus present in the soil in the chemosorbed plus easily-acid-soluble fraction.

The yields of carotene are also related to the amounts of phosphorus in this fraction (Figs. 11 and 12). Points 1 and 7 (Fig. 11) represent soils which contain very low and exceptionally high amounts of replaceable bases, respectively. The amount of replaceable bases evidently is more important than the amount of phosphorus in this form in governing the total yield of carotene in the leaves of oats and rye.

Easily-Acid-Soluble Phosphorus Fraction: The data (Figs. 13 and 14) show a vaguely positive relationship between the amounts of easily-acid soluble phosphorus in the soil and the concentration of carotene in the leaves. The data for rye is a little more convincing than that for oats.

No definite relationship is indicated (Figs. 15 and 16) to exist between the amounts of phosphorus present in the soil in this fraction and the total yields of carotene.

DISCUSSION

An examination of all the data presented shows that the concentrations of carotene in the leaves of oats and rye are positively correlated with the amounts of phosphorus present in the soil in the P_1, chemosorbed, and chemosorbed plus easily-acid-soluble fractions. These three fractions are

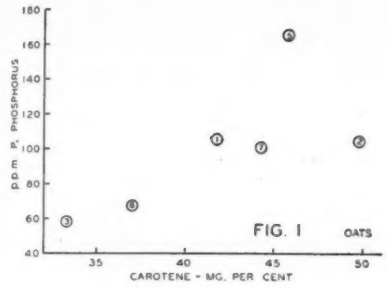


FIG. 1 OATS

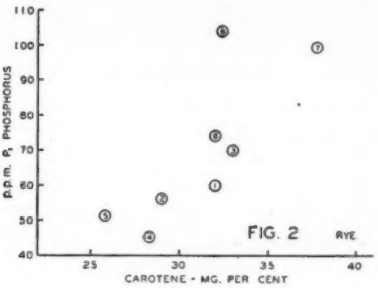


FIG. 2 RYE

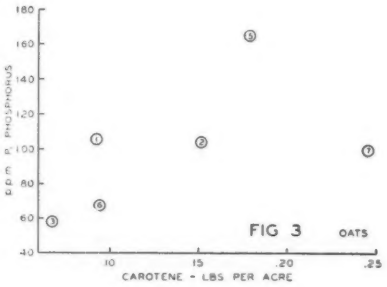


FIG 3 OATS

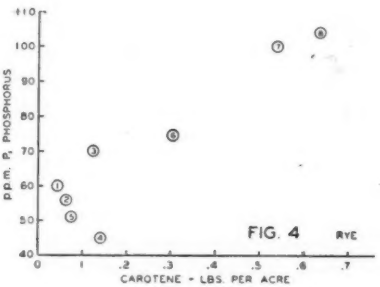


FIG. 4 RYE

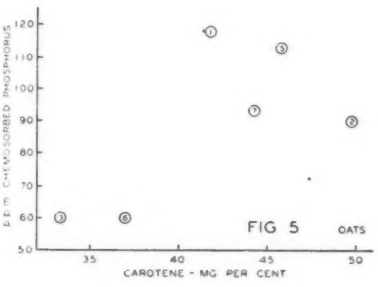


FIG 5 OATS

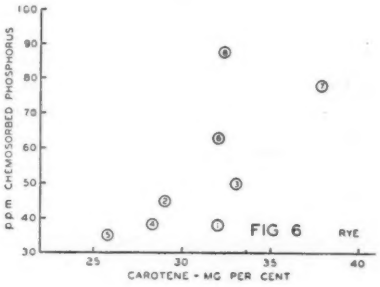


FIG 6 RYE

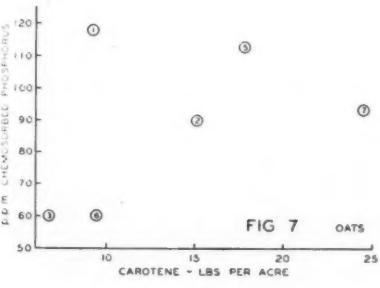


FIG 7 OATS

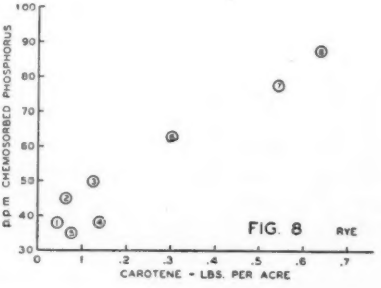
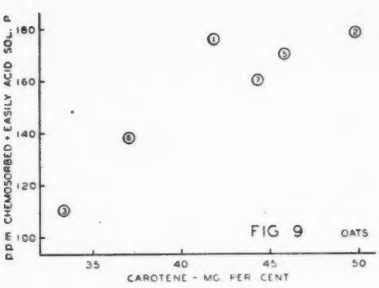
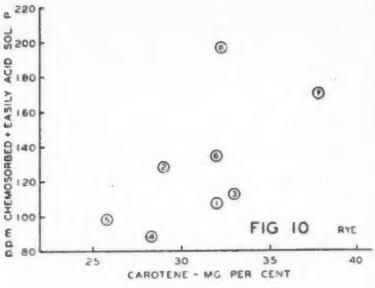
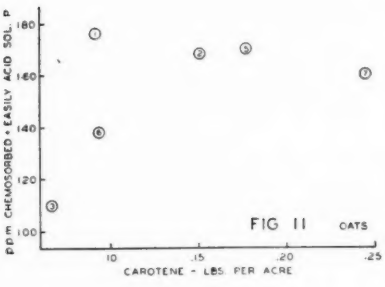
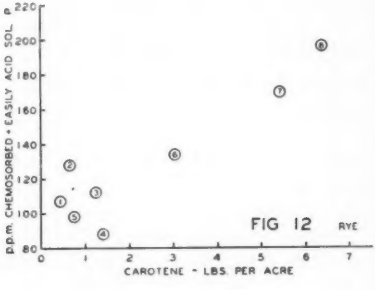


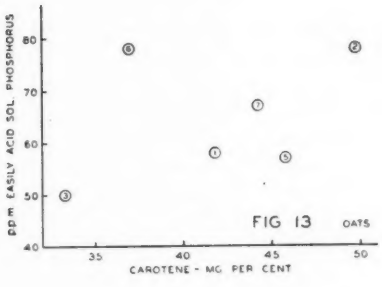
FIG. 8 RYE

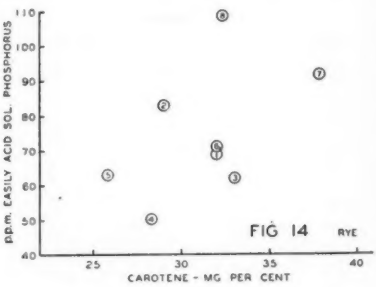

p.p.m. CHEMOSORBED + EASILY ACID SOL. P
CAROTENE - MG. PER CENT
FIG 9 OATS


p.p.m. CHEMOSORBED + EASILY ACID SOL. P
CAROTENE - MG. PER CENT
FIG 10 RYE


p.p.m. CHEMOSORBED + EASILY ACID SOL. P
CAROTENE - LBS. PER ACRE
FIG 11 OATS


p.p.m. CHEMOSORBED + EASILY ACID SOL. P
CAROTENE - LBS. PER ACRE
FIG 12 RYE


p.p.m. EASILY ACID SOL. PHOSPHORUS
CAROTENE - MG. PER CENT
FIG 13 OATS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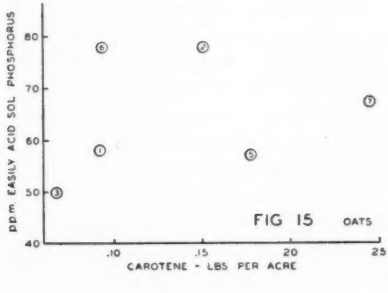
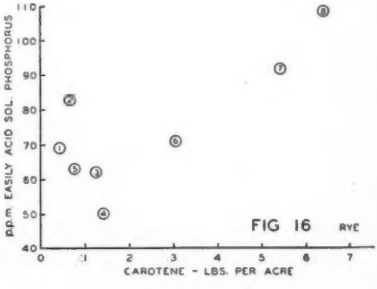
CAROTENE - MG. PER CENT
FIG 14 RYE


p.p.m. EASILY ACID SOL. PHOSPHORUS
CAROTENE - LBS. PER ACRE
FIG 15 OATS


p.p.m. EASILY ACID SOL. PHOSPHORUS
CAROTENE - LBS. PER ACRE
FIG 16 RYE

composed largely of chemosorbed phosphorus. Wynd and Noggle (1943) have pointed out that the magnitudes of these fractions are related to the amounts of organic matter and nitrogen in the soils studied. The base-exchange capacity, which depends largely on the organic matter in these soils, determines in many instances the amount of replaceable bases maintained in the soils, and therefore the amounts of replaceable bases also are related sometimes to the amounts of phosphorus present in these fractions.

The association between these chemical constituents in the soil makes it difficult or impossible to separate the individual effects of each of the components of the nutritional complex. This difficulty is especially great in the attempt to compare the effects of nitrogen and these three phosphorus fractions on the concentration of carotene in the leaves.

There is a vaguely positive relationship between the amount of easily-acid-soluble phosphorus and the concentration of carotene in the leaves. If this relationship be true, it is of especial significance because this fraction of phosphorus has been shown by Wynd and Noggle (1943) to be unrelated to the amount of organic matter or of nitrogen. A relationship between this fraction and the concentration of carotene therefore would show that phosphorus was more important than nitrogen in influencing the concentration of carotene in the leaves.

The total yields of carotene in pounds per acre also appears to be positively correlated with the amounts of soil phosphorus present in the P_1, chemosorbed, and chemosorbed plus easily-acid-soluble fractions. However, this correlation exists only if the higher amounts of phosphorus are accompanied by correspondingly higher amounts of replaceable bases. The amounts of replaceable bases are more important than the amounts of phosphorus because of the effect that they exert on the growth.

The yields of carotene do not appear to be influenced by the amounts of soil phosphorus present in the easily-acid-soluble condition. This situation is probably due to the fact that easily-acid-soluble phosphorus is not associated with the chemical factors which limit growth.

SUMMARY

The amounts of phosphorus present in each of four fractions designated as P_1, chemosorbed, chemosorbed plus easily-acid-soluble, and easily-acid-soluble were determined in 15 soils in the vicinity of Midland, Douglas County, Kansas. The concentrations of carotene in the leaves of oats and rye, grown on each of these soils and harvested at the jointing stage, were determined.

The concentrations of carotene in the leaves were positively related to the amounts of phosphorus in the soil in the P_1, chemosorbed, and chemosorbed plus easily-acid-soluble fractions, but this was due probably to the association of these fractions with organic nitrogenous material in the soil. The amount of nitrogen in the soils studied is probably more important than the amount of phosphorus in governing the concentration of carotene in the leaves.

The yields of carotene in pounds per acre are also positively related to the amounts of phosphorus present in the P_1, chemosorbed, and chemosorbed plus easily-acid-soluble fractions, but only if these amounts of

phosphorus are themselves positively correlated with the amounts of replaceable bases. The amounts of replaceable bases dominate the effect of phosphorus in determining the total yield of carotene because of the effect the bases exert on growth.

The amounts of easily-acid-soluble phosphorus exhibited a vaguely positive relation to the concentration of carotene in the leaves, but there was no correlation of this fraction with the total yield of carotene.

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RELATIONSHIP BETWEEN PHOSPHORUS FRACTIONS IN SOIL AND VITAMIN C PRODUCTION IN LEAVES OF CEREALS[1]

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(Received for publication, July 23, 1945)

The chemical properties of a group of soils in the vicinity of Midland, Douglas County, Kansas, have been described in detail by Wynd and Romig (1943). The associations which exist between the various phosphorus fractions and certain other chemical components in these soils have been reported by Wynd and Noggle (1943). These authors later described the relationships which existed between the amounts of phosphorus in these various fractions and the growth (1946a), the protein content (1946b), and the carotene content (1946c) of cereal leaves.

The purpose of the present paper is to report the relationships which exist between the amounts of soil phosphorus in various fractions and the amounts of vitamin C in the leaves of oats and rye. Very little is known about the effects of the nutritional conditions on the vitamin content of plants, and there is particularly little information available concerning the effect of cultural conditions on the vitamin C content of agriculturally important plants.

Unpublished data obtained by the authors show that the vitamin C content of cereal leaves is more variable and depends more sensitively on factors in the environment than do most vitamins. For example, some sets of data show that the plants showing greatest growth and producing the highest yields of dry matter contain the greatest concentration of vitamin C. Other data show that exactly the opposite relationship is true, while still other sets of data fail to show that the concentration of vitamin C bears any relationship at all to the growth rate or to the yield of dry matter. These studies indicate that some subtle, and hitherto unrecognized, factor or factors influence the production of this vitamin in plant tissue. The extensive use that is now being made of immature cereal leaves as sources of vitamins lends especial importance to the study of the conditions which affect the concentration of these substances and their yields in plants.

MATERIALS AND METHODS

Oats were grown on six fields and rye was grown on eight fields in the vicinity of Midland, Douglas, County, Kansas. The crops were harvested just as jointing began because the concentration of vitamins in the leaves is particularly high during this stage of physiological development. The material was quickly dried in an Arnold hot-air dehydrator.

The vitamin C was determined on the same samples that furnished the previously published data on the growth (1946a), protein content (1946b), and carotene content (1946c) of the leaves. The amount of vitamin C was

[1] The expenses incurred by the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

TABLE 1

Phosphorus Fractions in Soils[1]

M.S.C. file No.	Field No.	Point No.	Phosphorus fractions			
			P_1	Chemo-sorbed	Chemo-sorbed plus easily-acid-soluble	Easily-acid-soluble
			p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
267	2A	1	105	118	176	58
268	2B	2	104	90	168	78
269	19	3	58	60	110	50
270	11E	4	76	63	95	32
272	12	5	165	113	170	57
274	14B	6	67	60	138	78
280	18	7	100	93	160	67
286	9	1	60	38	107	69
285	10	2	56	45	128	83
288	20	3	70	50	112	62
284	19B	4	45	38	88	50
287	22	5	51	35	98	63
291	11	6	74	63	134	71
289	15A	7	100	78	170	92
290	15B	8	104	88	197	109

[1] The first group of points, 1 to 7 inclusive, refers to soils planted with oats; the second group of points, 1 to 8 inclusive, refers to soils planted with rye.

TABLE 2

Concentration and Yield of Vitamin C in Cereal Leaves[1]

M.S.C. file No.	Field No.	Point No.	Date harvested	Yield of dry matter per acre	Vitamin C	Vitamin C per acre
				lb.	*mg./100 gm.*	*lb.*
315	2A	1	Oct. 8, 1940	221	425	0.939
317	2B	2	Oct. 11, 1940	304	486	1.477
319	19	3	Oct. 17, 1940	200	389	0.778
320	11E	4	Oct. 18, 1940	200	.....	.....
321	12	5	Oct. 21, 1940	389	472	1.836
318	14B	6	Oct. 23, 1940	253	361	0.913
332	9	1	Apr. 10, 1941	144	372	0.54
334	10	2	Apr. 11, 1941	221	338	0.75
335	20	3	Apr. 12, 1941	379	387	1.47
336	19B	4	Apr. 13, 1941	500	291	1.46
337	22	5	Apr. 14, 1941	298	291	0.87
338	11	6	Apr. 17, 1941	947	226	2.14
339	15A	7	Apr. 20, 1941	1430	274	3.92
340	15B	8	Apr. 20, 1941	1950	274	5.40

[1] The first group of points, 1 to 6 inclusive, refers to soils planted with oats; the second group of points, 1 to 8 inclusive, refers to soils planted with rye.

determined by triturating one gram of the dried, powdered sample in a mortar with 25 milliliters of an extracting solution which contained two per cent of metaphosphoric acid and three per cent of trichloracetic acid. One milliliter of the filtrate was then titrated with a standard solution of 2,3-dichlorophenolindophenol.

The data on the phosphorus fraction in the soil were obtained by the procedure of Bray and his co-workers. These methods have been reviewed in detail by Wynd and Noggle (1943). The data concerning the phosphorus fractions are shown (Table 1). Further details of the chemical properties of the soils may be obtained by comparing the corresponding University of Illinois file numbers in the tables previously published, Wynd and Romig (1943). All comments in the text concerning chemical characteristics not specifically reported in the present paper are based on the data in those tables.

The data for the vitamin C content of the leaves appear (Table 2); the point numbers enable the reader to identify in Table 1 the individual soils from which each of the harvests indicated in the figures was obtained.

EXPERIMENTAL RESULTS

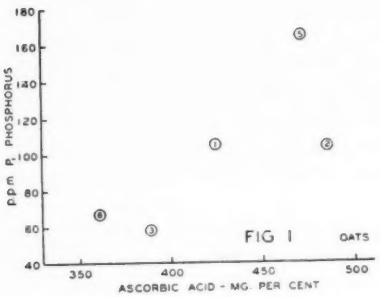
P_1 Phosphorus Fraction: The data (Fig. 1) show that the amount of P_1 phosphorus in the soil is positively related to the concentration of vitamin C in the leaves. Points 1, 2, and 5 represent dark-colored soils containing from 0.070 to 0.135 per cent of nitrogen. This group of three soils contained the highest amount of P_1 phosphorus and they produced the highest concentration of vitamin C in the leaves. The points in Fig. 2 fall into two groups—Points 6 to 8 and Points 1 to 5, inclusive. The first group represents soil rich in nitrogen, while the latter represents soils poor in nitrogen. Within each group the amounts of P_1 phosphorus are positively related to the concentration of vitamin C, but the group of richer soils produced a smaller concentration of vitamin C in the leaves than did the poor soils. It is apparent that the concentration of vitamin C is associated not only with the amount of P_1 phosphorus but also with other properties of the soil groups. Comparison (Figs. 1 and 2) shows that these other conditions may or may not be associated with the P_1 fraction of phosphorus.

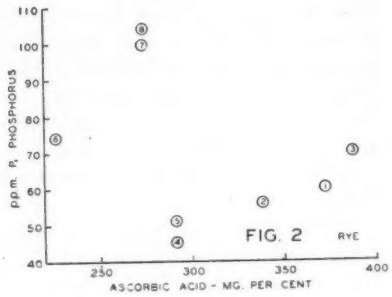
The yields of vitamin C in pounds per acre are closely related to the magnitudes of the P_1 phosphorus fraction (Fig. 3). The facts that soils represented by Points 1, 6, and 3 are rich in nitrogen and replaceable bases, and Points 3 and 6 represent comparatively poor soils, do not disturb the relationship between this phosphorus fraction and the yield. The data (Fig. 4) show that a similar positive relationship exists for the soils richer in nitrogen and bases but that exactly the opposite is true for the poorer soils.

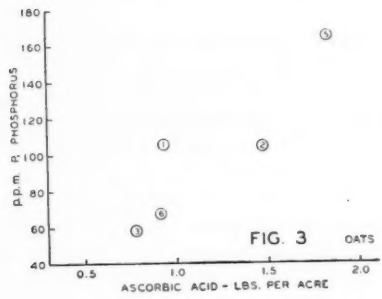
Chemosorbed Phosphorus Fraction: The concentrations of vitamin C in the leaves of oats are positively related to the amounts of chemosorbed phosphorus (Fig. 5). This positive relationship is true also for rye grown on the richer soils represented by Points 6, 7, and 8 (Fig. 6). A positive relationship exists with the group of poorer soils indicated by Points 1 to 5, inclusive, but just as in the case of P_1 phosphorus, it is again apparent that

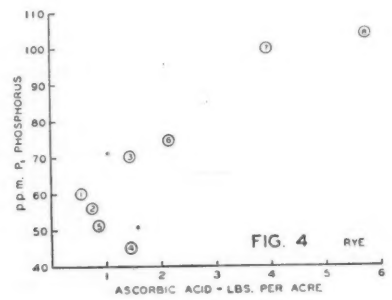
some other soil property is important in controlling the concentration of vitamin C in the leaves of rye.

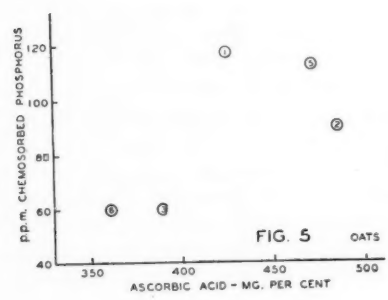
The data presented (Figs. 7 and 8) show that the yields of vitamin C by both oats and rye are positively related to the magnitudes of this phosphorus fraction.

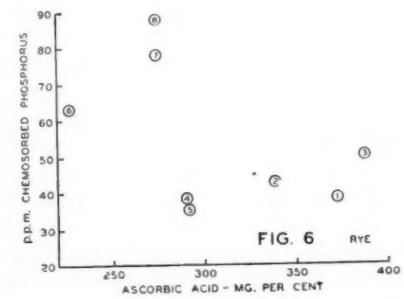






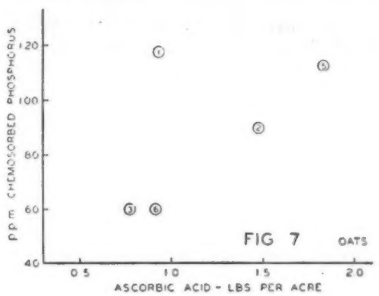


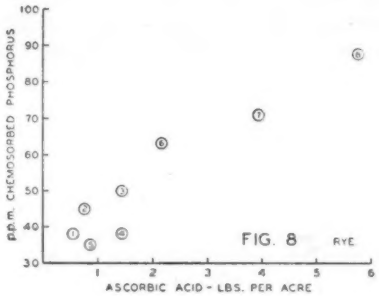


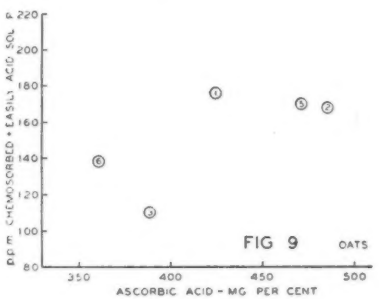


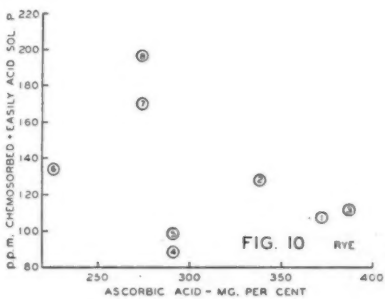
Chemosorbed Plus Easily-Acid-Soluble Phosphorus Fraction: The data presented (Fig. 9) show that the high concentrations of vitamin C in the leaves of oats were obtained from soils which contained the greatest amounts of phosphorus in the chemosorbed plus easily-acid-soluble fraction. Figure 10 is more difficult to interpret. It is, however, evident that the soils rich in nitrogen and bases, represented by Points 6, 7, and 8,

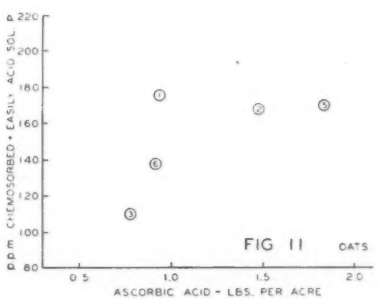
were inferior to the poorer soils represented by Points 1 to 5, inclusive, in respect to the concentration of vitamin C in the crops. Each of these groups, however, shows a positive relationship between the amounts of phosphorus in this fraction and the concentration of vitamin C in the leaves of rye.

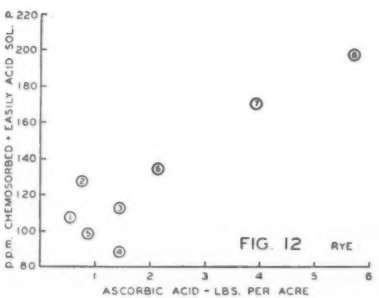








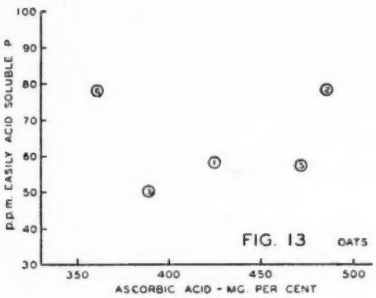


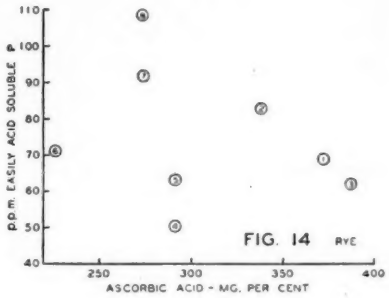


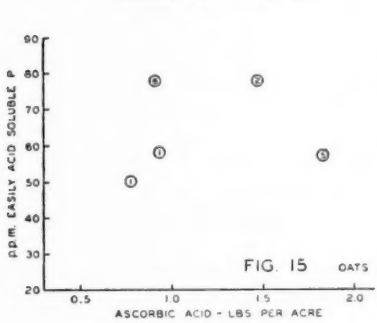
The total yields of vitamin C in pounds per acre are shown (Figs. 11 and 12) to be positively related to the amount of phosphorus in the chemosorbed plus easily-acid-soluble fraction.

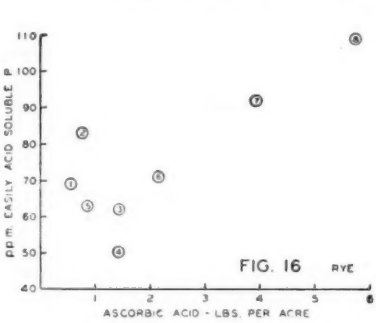
Easily-Acid-Soluble Phosphorus Fraction: It is difficult to recognize from Figs. 13 and 14 any relationship between the amounts of easily-acid-soluble phosphorus in the soil and the concentration of vitamin C in the leaves of oats and rye.

A generally positive relationship is indicated (Fig. 15) between the amounts of phosphorus in this fraction and the yield of vitamin C. Point 5 represents a soil very rich in replaceable bases from which a large yield of dry matter was obtained; consequently the yield of vitamin C was also exceptionally large. The yields of vitamin C from the poorer soils represented by Points 1 to 5, inclusive, (Fig. 16) are inversely related to the amounts of easily-acid-soluble phosphorus in the soil, while the opposite is true for the richer soils represented by Points 6 to 8, inclusive.









DISCUSSION

The data presented in the tables and figures show that there is a positive relationship between the concentrations of vitamin C in the leaves of oats and rye and the amounts of soil phosphorus present in the P_1, chemosorbed, and chemosorbed plus easily-acid-soluble fractions. Wynd and Noggle (1943) have pointed out that these three fractions are composed largely of chemosorbed phosphorus and, further, that the amounts of chemosorbed phosphorus are directly proportional to the amounts of organic matter in the soil. Since the amounts of organic matter in the soils studied govern also the magnitudes of their base-exchange capacities, the amounts of replaceable bases retained in the soil are more or less proportional to the amounts of organic matter and are associated therefore with chemosorbed phosphorus. It is impossible to state that any one component of this complex of nutritional factors is specifically responsible for the observed crop responses. The statements in the text that certain soil fac-

tors are associated with certain crop responses do not imply that these factors necessarily control the crop responses.

Even though chemosorbed phosphorus appears to be related to the concentration of vitamin C in the leaves, it is apparent from the grouping of the points (Figs. 2, 6, and 10) that there are other important factors peculiar to the type of soil studied.

The easily-acid-soluble phosphorus does not appear to be related to the concentration or to the yield of vitamin C in the leaves of cereals. This fraction has been shown by Wynd and Noggle (1943) to be independent of the organic matter and of the nutritional factors associated with it.

The total yields of vitamin C are governed by the yields of dry matter as well as by the concentration of the vitamin in the tissue. The yield of dry matter is the more important of these factors.

The intricate system of relationships which exists between the nutritional factors of the soil leads the student of plant nutrition into serious errors in interpretation if only single factors are studied in respect to plant response.

SUMMARY

The amounts of soil phosphorus present in each of the fractions designated as P_1, chemosorbed, chemosorbed plus easily-acid-soluble, and easily-acid-soluble were determined in 14 soils in the vicinity of Midland, Douglas County, Kansas. The concentrations of vitamin C in the leaves of oats and rye, grown on each of these soils and harvested at the jointing stage, were determined.

The concentrations of vitamin C in the leaves were positively related to the amounts of phosphorus in the soil in the P_1, chemosorbed, and chemosorbed plus easily-acid-soluble fractions. Other unrecognized properties, peculiar to the kind of soil, were also associated with the concentration of vitamin C in the leaves.

The yields of vitamin C in pounds per acre were positively associated with the amounts of phosphorus in each of the four fractions studied although this was less evident for the easily-acid-soluble fraction. These relationships were true only when the amounts of replaceable bases were proportional to the amounts of phosphorus.

The total yields of vitamin C are more dependent on the yields of dry matter than upon the concentrations of the vitamin in the tissue.

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INFLUENCE OF NUTRITIONAL FACTORS ON CORRELATION BETWEEN VITAMIN C CONCENTRATION AND GROWTH OF CEREALS[1]

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(Received for publication, July 23, 1945)

The concentrations of vitamin C in plant tissue vary between wide limits. The concentrations of this vitamin have been found to vary from 200 to 700 milligrams per 100 grams in the leaves of cereal plants at the shooting stage, calculated on the dry-weight basis.

Wynd and Noggle (1945) have reported that the concentration of vitamin C in the leaves of oats grown near Midland, Douglas County, Kansas, was directly proportional to the nitrogen content of the soil. The yield of dry matter, harvested at the jointing stage, was also directly proportional to the concentration of vitamin C in the leaves. This positive correlation between the yield of dry matter and the concentration of vitamin C suggests that the plants growing more rapidly contain the higher concentration of this vitamin. Yet, data reported by Wynd and Noggle (1946) obtained for rye grown in the same general area showed that sometimes exactly the opposite relationship existed.

The fact that the concentration of vitamin C is sometimes positively and sometimes negatively correlated with the yield of dry matter shows that the concentration of this vitamin does not depend intrinsically on the growth rate of the plant. The purpose of the present study was to determine if the nature of the nutritional complex present in the soil was able to influence the relationship between the yield of dry matter of oats and rye and the concentration of vitamin C in the leaves. The importance of immature cereal leaves as a source of vitamin for stock feeds and for human foods lends a practical, as well as a theoretical, importance to the study of the factors influencing the accumulation of vitamins in their leaves.

METHODS AND MATERIALS

Oats were grown on five soils in the vicinity of Midland, Douglas County, Kansas, in the autumn of 1940, and rye was grown on eight soils in the same general area during the spring of 1941. The crops were harvested just as shooting began, since the concentration of vitamin C in the leaves is especially high at this stage of physiological development. The tissue was quickly dried in an Arnold hot-air dehydrator, and the vitamin C determined by titration as described by Wynd and Noggle (1945).

Samples of the upper eight inches of soil were collected from each field when the crop was harvested. The chemical properties of the soil were determined by the methods described by Noggle and Wynd (1941), Wynd and Romig (1943), and Wynd and Noggle (1943).

[1] The expenses incurred by the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

The data for vitamin C are presented (Table 1); the M. S. C. file numbers refer to the original samples of leaf tissue preserved in the Department of Botany and Plant Pathology, Michigan State College. The point numbers refer to the individual data as they appear (Fig. 1).

TABLE 1

Yield of Dry Matter and Concentration of Vitamin C in Leaves of Oats

M. S. C. file No.	Field No.	Date harvested	Point No.[1]	Dry matter per acre	Vitamin C
				lb.	*mg./100 gm.*
315	2A	Oct. 8, 1940	1	221	425
317	2B	Oct. 11, 1940	2	304	486
319	9	Oct. 17, 1940	3	200	389
321	2	Oct. 21, 1940	4	389	472
318	14B	Oct. 23, 1940	5	253	361
332	9	Apr. 10, 1941	1	144	372
334	10	Apr. 11, 1941	2	221	338
335	20	Apr. 12, 1941	3	379	387
336	19B	Apr. 13, 1941	4	500	291
337	22	Apr. 14, 1941	5	298	291
338	11	Apr. 17, 1941	6	947	226
339	15A	Apr. 20, 1941	7	1430	274
340	15B	Apr. 20, 1941	8	1970	274

[1] The first group of points, numbered 1 to 5 inclusive, refers to oats; the second group of points, numbered 1 to 8 inclusive, refers to rye.

The data for the chemical components in the soil are presented (Tables 2 and 3); the relationships between three groups of harvests are represented graphically (Fig. 1), and the influence of soil properties (Figs. 2 to 12), inclusive. In this latter group of 11 figures the relative magnitudes of the various nutritional factors are presented. These values were calculated by setting the highest magnitude of the factor under consideration equal to 100. The solid bars represent fields which produced crops exhibiting a positive relationship between growth and vitamin C concentration, and the broken bars represent fields which produced crops exhibiting a negative correlation between growth and the concentration of vitamin C. These fields will be referred to as "positive" and "negative," respectively. The numbers along the abscissa of these figures refer to the identification numbers of the fields studied.

EXPERIMENTAL RESULTS

The upper graph (Fig. 1) shows that the yields of the dry matter of oats obtained during the autumn of 1940 were positively related to the concentration of vitamin C in the leaves. The center graph shows that this positive relationship also was observed for three harvests of rye grown in different soils during the spring of 1941. The lower graph shows that there was a negative correlation between growth and vitamin C concentration in rye harvested in the spring of 1941 from a different group of soils.

The negative soils all exhibited a low loss on ignition (Fig. 2). Reference to the data (Table 2) discloses that the division between the positive and negative soils comes between 2.31 and 2.19 percentage loss on ignition.

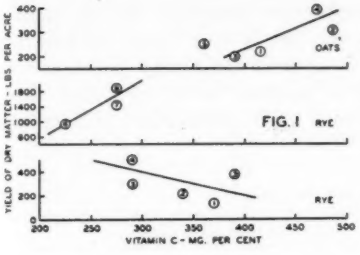

FIG. 1

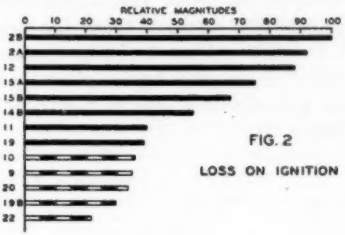

FIG. 2
LOSS ON IGNITION

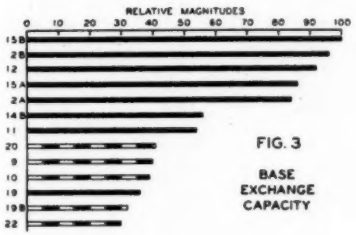

FIG. 3
BASE EXCHANGE CAPACITY

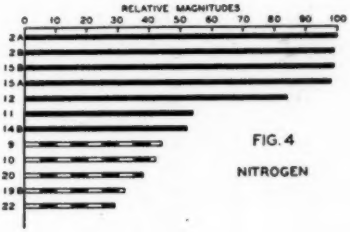

FIG. 4
NITROGEN

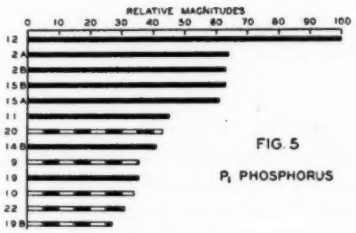

FIG. 5
P_i PHOSPHORUS

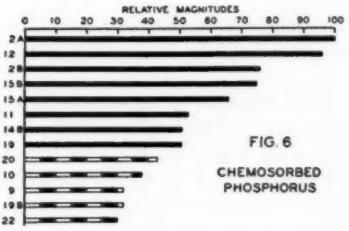

FIG. 6
CHEMOSORBED PHOSPHORUS

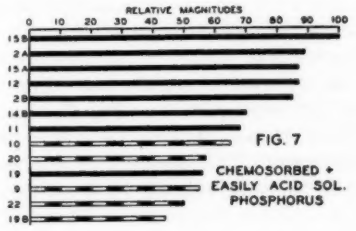

FIG. 7
CHEMOSORBED + EASILY ACID SOL. PHOSPHORUS

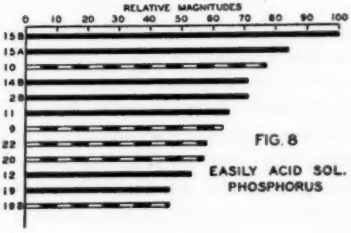

FIG. 8
EASILY ACID SOL. PHOSPHORUS

In general, the negative soils exhibited a low base-exchange capacity (Fig. 3), a fact which is due to the low amount of organic matter present. The magnitudes of the base-exchange capacities, however, do not clearly separate the positive from the negative soils, as is seen from the location of Field 19 (Fig. 3). The positive and negative soils may be definitely separated on the basis of their nitrogen content (Fig. 4); data show that this division occurs between 0.070 and 0.058 percentage of nitrogen (Table 2).

TABLE 2

Organic Matter Complex in Soils

M. S. C. file No.	Field No.	Point No.[1]	Organic-matter complex		
			Loss on ignition	Base-exchange capacity	Nitrogen
			pct.	*me.*	*pct.*
267	2A	1	5.35	20.8	0.137
268	2B	2	5.84	24.0	0.135
269	19	3	2.31	9.1	0.047
272	12	4	5.13	22.9	0.115
274	14B	5	3.22	13.9	0.070
286	9	1	2.03	9.9	0.058
285	10	2	2.19	9.7	0.057
288	20	3	2.02	10.3	0.052
284	19B	4	1.78	8.1	0.042
287	22	5	1.57	7.6	0.040
291	11	6	2.32	13.5	0.071
289	15A	7	4.36	21.6	0.133
290	15B	8	3.94	25.0	0.135

[1] The first group of points, numbered 1 to 5 inclusive, represents soils planted with oats; the second group of points, numbered 1 to 8 inclusive, represents soils planted with rye.

The amount of phosphorus present in the P_1 fraction furnishes but slight basis for the separation of the positive from the negative soils (Fig. 5); however, the magnitude of the chemosorbed phosphorus fraction is shown (Fig. 6) to permit an accurate separation of the soils to be made. This division comes between 60 and 50 parts per million of chemosorbed phosphorus. The amount of phosphorus in the chemosorbed plus easily-acid-soluble fraction also permits a moderately good separation of positive from negative soils. Soil 19 is, however, an exception. The magnitude of the easily-acid-soluble phosphorus fraction is shown (Fig. 8) to bear no relation to positive and negative groups of soils.

The positive and negative soils may be separated roughly according to the amount of replaceable bases present, although Soil 19 (Fig. 9) is an exception. A similar situation exists (Figs. 10 and 11) in regard to the amounts of replaceable calcium and magnesium. The amounts of replaceable potassium, on the other hand, are in no way related to positive or negative character of the soils (Fig. 12).

DISCUSSION

A survey of all the data presented in the figures shows that a perfect separation of positive from negative soils may be made on the basis of the percentage loss on ignition and on the amount of nitrogen and chemo-

sorbed phosphorus present. It is to be expected that the magnitude of these three soil properties would be related in a similar manner to the response of the plant since Wynd and Romig (1943) have shown that the amounts of nitrogen in these soils are quantitatively related to the amounts of organic matter present, and Wynd and Noggle (1943) have found that the amounts of phosphorus present in the chemosorbed fraction are also proportional to the amounts of organic matter. On the basis of the data at hand, it is not possible to separate the effects of nitrogen and chemosorbed phosphorus in determining the positive and negative character of the soils studied.

TABLE 3

Replaceable Bases and Phosphorus Fractions in Soils[1]

M. S. C. file No.	Replaceable bases (milliequivalents per 100 grams)				Phosphorus fractions			
	Total	Ca	Mg	K	P_1	Chemosorbed	Chemosorbed, easily-acid-soluble	Easily-acid-soluble
					p. p. m.	*p. p. m.*	*p. p. m.*	*p. p. m.*
267	18.0	16.8	3.2	0.63	105	118	176	58
268	20.9	19.3	3.6	0.52	104	90	168	78
269	14.0	13.2	1.3	0.47	58	60	110	50
272	32.7	30.4	2.8	0.98	165	113	170	57
274	19.8	18.4	1.7	0.81	67	60	138	78
286	11.6	10.7	1.5	0.60	60	38	107	69
285	14.4	13.5	1.5	0.63	56	45	128	83
288	14.8	14.3	1.6	0.53	70	50	112	62
284	12.6	12.2	1.3	0.25	45	38	88	50
287	10.3	9.6	1.1	0.27	51	35	98	63
291	22.0	20.2	2.2	0.55	74	63	134	71
289	18.0	17.8	2.9	0.55	100	78	170	92
290	23.7	21.2	3.3	0.82	104	88	197	109

[1] For point numbers, field numbers, and crops, refer to Table 2 under entries bearing similar M. S. C. file numbers.

The complex of nutritional factors associated with the base-exchange capacity of the soil also are associated, to a greater or less degree, with the organic matter present, since Wynd and Romig (1943) have shown that the magnitude of the base-exchange capacity depends largely on the amount of organic matter in these soils. This association between the amount of organic matter and the magnitude of the base-exchange capacity is not true, however, in all the soils studied, as is apparent from a comparison of the relative position of Soil 19 (Figs. 2 and 3). The amounts of replaceable bases appear to be related to the positive or negative character of the soils in most instances, but this is true *only* if their amounts are proportional to the amount of organic matter present. Hence, the amounts of total replaceable bases, calcium, and magnesium also are correlated with the positive or negative character of the soils in many instances. The amounts of replaceable potassium, on the other hand, show no such relationship.

The various fractions of soil phosphorus studied appear to be related to the positive or negative character of the soils, but just as in the in-

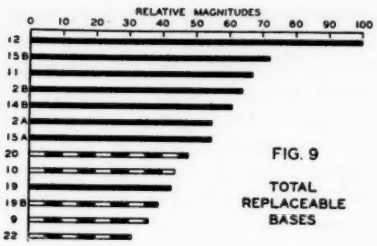

FIG. 9

TOTAL REPLACEABLE BASES

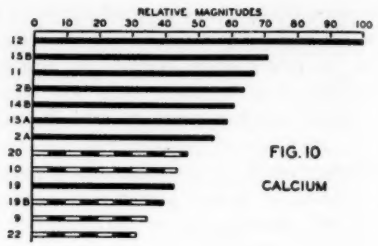

FIG. 10

CALCIUM

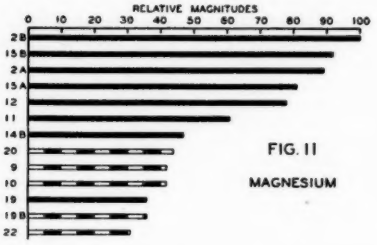

FIG. 11

MAGNESIUM

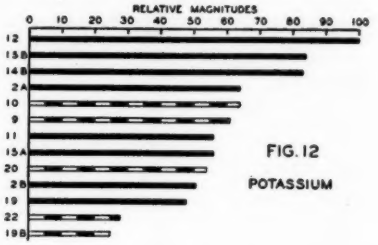

FIG. 12

POTASSIUM

stances of the replaceable bases, this relationship is true *only* if their magnitudes are proportional to the amounts of organic matter present. Hence, there is perfect relationship between the chemosorbed phosphorus fraction to the positive or negative character of the soils, a slightly less predictable relationship of the chemosorbed plus easily-acid-soluble fraction, a still lesser degree of relationship of the P_1 fraction, and finally no relationship at all between the easily-acid-soluble fraction and the positive or negative character of the soils.

On the basis of the present data, the positive and negative soils may be separated according to the numerical magnitudes of three components of the nutritional complex, all of which are associated with the organic matter. The critical values of these properties are as follows: loss on ignition from 2.31 to 2.19 per cent, nitrogen from 0.070 to 0.058 per cent, and chemosorbed phosphorus from 60 to 50 parts per million.

It must be emphasized that the critical value of the factors listed above were obtained from the study of a particular group of soils in the vicinity of Midland, Douglas County, Kansas, under the climatic conditions which existed during the autumn of 1940 and spring of 1941. It is not known how widely applicable these values may be and especially how their magnitudes depend on soil type and climate. It is important, however, to know that critical values of certain soil nutritional factors do exist which govern the ability of cereal plants to accumulate vitamin C in their leaves in amounts proportional to their rate of growth.

SUMMARY

A group of 13 soils in the vicinity of Midland, Douglas County, Kansas, were studied in respect to their base-exchange capacity and their content

of organic matter, nitrogen, total replaceable bases, replaceable calcium, magnesium, potassium, and the phosphorus present in fractions designated as P_1, chemosorbed, chemosorbed plus easily-acid-soluble, and easily-acid-soluble. The yields of dry matter of oats and of rye, grown on these soils and harvested at the jointing stage, and the concentration of vitamin C in the leaves were determined.

A group of eight of the soils produced cereal crops which exhibited a positive correlation between yield of dry matter and concentration of vitamin C, while a group of five soils produced crops which exhibited a negative correlation. The magnitude of the nutritional factors associated quantitatively with the organic matter in the soil determined the positive or negative nature of the correlation between yield of dry matter and concentration of vitamin C in the leaves.

Soils which permitted a positive correlation between the yield of dry matter and concentration of vitamin C were characterized by nutritional factors having at least the following magnitudes: loss on ignition 2.31 to 2.19 per cent, nitrogen 0.070 to 0.058 per cent, and chemosorbed phosphorus 60 to 50 parts per million.

The amounts of total replaceable bases, replaceable calcium and magnesium, and the amount of phosphorus present in the P_1 and chemosorbed plus easily-acid-soluble fractions in the soils were also related to the correlation between the yield of dry matter and concentration of vitamin C in the leaves, but this was true *only* if the magnitudes of these nutritional factors were proportional to the amount of organic matter.

The amounts of replaceable potassium and easily-acid-soluble phosphorus in the soils were not related to the correlation of the yields of dry matter and concentrations of vitamin C. The magnitudes of these two factors also were not related to the amount of organic matter in the soils.

It is not known how the magnitudes of the critical values are affected by soil type or by climate, but it is important to know that certain nutritional properties of the soil do exist which govern the ability of cereals to accumulate vitamin C in the leaves in amounts proportional to the growth rate.

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AN INVESTIGATION OF A SOURCE AND MODE OF ENTRY OF *SALMONELLA* ORGANISMS IN SPRAY-DRIED WHOLE-EGG POWDER

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Microbiological examination of more than 5,000 samples of spray-dried whole-egg powder (94 to 96 per cent total solids content) manufactured in the United States between September 1, 1943, and January 1, 1945, has revealed a relatively high incidence of *Salmonella* contamination. Gibbons and Moore (1944) have reported the presence of *Salmonella* organisms in spray-dried whole-egg powder manufactured in Canada between November, 1942 and December, 1943. Inasmuch as the presence of such organisms in food products is highly undesirable, an investigation was undertaken to determine their source and mode of entry into the powder.

In view of the nature of the dehydration process, it would seem that the *Salmonella* organisms enter the processing system by way of the raw material. If such is the case, they may be introduced into the egg at the time of its formation within the hen or deposited on the shell at the time of laying or by later contact with fecal or soil material. Several investigators, including Rettger (1913), Hadley and Caldwell (1916), Bryant and Sharp (1934), and Stuart and McNally (1943), have observed that egg meats of clean shell eggs are generally sterile. Jenkins, Hepburn, Swan, and Sherwood (1920), Bryant and Sharp (1934), and others have reported that after storage a larger percentage of dirty eggs contain bacteria than do clean eggs. The latter investigators also reported that eggs heavily coated with manure that had been allowed to dry overnight contained bacteria after storage, regardless of the washing methods used. Haines (1938), in his study on the bacterial flora on egg shells, found that shells soiled with feces had a greater number of bacteria than the shells of clean eggs. Microorganisms derived from feces, manure, and soil may penetrate through the shell and shell membrane and develop within the egg meat if temperature and humidity conditions are favorable. Scott (1930) and Brown, Combs, and Wright (1940) have demonstrated the penetration of *S. aertrycke* through the shell. Rettger (1913), Schalm (1937), Haines and Moran (1940), Stuart and McNally (1943), Mallmann

and Davidson (1944), and others have demonstrated penetration of a variety of microorganisms through the egg shell. Environmental conditions under which eggs are handled today on farms, in stores, in produce houses, in cold-storage houses, and at dehydration plants frequently are favorable for shell penetration.

These studies were concerned primarily with investigating the presence of *Salmonella* in the raw material as received and used in egg-dehydration plants. The data suggest a mode of entry of the organisms into the processing system and ultimately into the finished product.

Field studies were undertaken in six representative dehydration plants during the months of February and March, 1944, and again in August, September, and October of the same year. Field laboratories were set up in two plants, one in Texas, the other in Illinois. Most of the supplies and equipment were furnished by the Mobile Laboratory, a field unit operated by the War Food Administration. The plants met the operational requirements of the War Food Administration (1943, 1944). They produced powder of four to six per cent moisture content and operated without preheating or pasteurization units. They differed in certain details but usually only where production capacity or physical requirements of space, etc., necessitated operational variation.

CULTURAL METHODS

Unless otherwise stated, the material to be examined for the presence of *Salmonella* was inoculated into 50 ml. of Selenite-F enrichment medium (B-B-L), incubated for 18 to 24 hours at 37°C.(98.6°F.) and then streaked out on two selective plate media, *Shigella-Salmonella* (SS) agar (Difco) and bismuth sulfite agar (Difco). The latter medium was included as the authors found it to be excellent for *Salmonella* isolations, particularly for *S. pullorum*. Suspected colonies appearing on the SS plates after 18 to 24 hours' incubation and on the bismuth sulfite plates after 42 to 48 hours' incubation were transferred to Kligler's iron agar (Difco) slants. After 18 to 24 hours' incubation, the slants were examined for evidence of hydrogen sulfide and gas production as well as for fermentation of lactose. Cultures conforming to the characteristic picture for *Salmonella* were grouped according to their "O" antigens with group antisera (B, C_1, C_2, D, E) by means of the macroscopic slide agglutination technic. Suspected positive cultures were returned to the laboratory of the Microbiology Research Division, Eastern Regional Research Laboratory, Philadelphia, for further study. It was soon found that microorganisms of the genera *Aerobacter, Proteus, Pseudomonas,* and *Escherichia* were recovered with considerable frequency among the cultures assumed to be *Salmonella.* Therefore, the following additions were made to the routine field procedure: Suspected colonies picked from the selective media plates were inoculated into tryptose broth for subsequent inoculations to differential media and incubated for 18 to 24 hours. From the tryptose broth, transfers were made to meat extract broth containing 0.5 per cent lactose and sucrose, respectively, to Kligler's iron agar slants, and to gelatin. The broth remaining after these inoculations were made was tested for the production of indol. This expanded preliminary differential analysis yielded

cultures characteristic of the *Salmonella* species. Stock cultures were carried on tryptose agar slants which, on arrival at the Eastern Regional Research Laboratory, were purified by streaking out on SS agar. Picked single colonies were tested for motility (semisolid agar and hanging drop), hydrogen sulfide and indol production, fermentation in lactose and sucrose broths, and liquefaction of gelatin. Fermentations of the purified cultures were also tested in meat extract broth containing 0.5 per cent dextrose, salicin, maltose, mannitol, arabinose, xylose, inositol, and sorbitol, respectively. The "O" antigens were checked, and the suspected cultures were sent to Temple University School of Medicine for serological identification. Methods employed in *Salmonella* typing were similar to those of Edwards and Bruner (1942).

SALMONELLA CONTAMINATION IN AND ON SHELLS, IN SHELL MEMBRANES, AND IN MEATS OF CLEAN, DIRTY, AND WASHED-DIRTY SHELL EGGS

Source of Material and Method of Sampling: Representative shell eggs from the daily allotment of eggs to be broken out were selected and examined for the presence of *Salmonella* organisms. Each egg was immersed in 50 ml. of Selenite-F for 30 to 60 minutes with periodic agitation, after which the Selenite-F wash from each egg was poured into another sterile container and incubated at 37°C. for 18 to 24 hours. The egg was then air dried, and the shell area over the air cell was swabbed with 70 per cent alcohol and opened aseptically. Either the whole egg meat was drained into a sterile container or the albumen and yolk were separately drained into sterile containers. Each was thoroughly mixed, and all or a portion of the material was inoculated into Selenite-F and incubated at 37°C. for 18 to 24 hours. The shell membrane was removed with a sterile forceps and transferred to a container of Selenite-F. Membranes from three or more eggs were introduced into the same container. The remaining shell was then broken into small pieces with a sterile mortar and pestle, partly ground, transferred to 50 ml. of Selenite-F, and incubated at 37°C. for 18 to 24 hours. The procedure for the examination of the incubated Selenite-F material has been described in the preceding section.

Results: Data obtained in this study are presented (Table 1). *Salmonella* organisms were recovered from the shell surfaces of three (one per cent) of 222 clean intact shell eggs, 11 (five per cent) of 232 dirty, and six (five per cent) of 123 washed-dirty shell eggs individually examined. "Carry-over" contamination in the laboratory by handling was probably not a factor. This is evidenced by the fact that in one series of 12 eggs examined on the same day, three different *Salmonella* types were recovered from three different Selenite-F shell washings. None of the remaining nine shell washings were positive for *Salmonella*.

From the ground and composited shell samples, *Salmonella* organisms were recovered from two per cent of the clean shells, 16 per cent of the dirty shells, and 13 per cent of the samples of the washed-dirty shells. Although the few contaminated individual shells may have been uniformly distributed in the different composite samples, it seems more likely that the higher percentage of contamination in the composited samples was due to the recovery of *Salmonella* from the pores and internal surfaces of the shell as well as from the external surfaces.

Examination of the data (Table 1) concerning composited samples of shell membranes and egg meats from the individual shell eggs under consideration reveals the same relationship with respect to incidence of *Salmonella* contamination, that is, lower for the clean eggs and higher for the dirties and washed-dirties. Three composite samples of whole-egg meats positive for *Salmonella* were obtained from eggs whose composited shells and shell membranes also yielded *Salmonella*. In one instance, however, *S. pullorum* was recovered from the yolk of a dirty egg but could not be recovered from the albumen, membrane, shell surface, or ground shell.

TABLE 1

Salmonella Contamination in and on Shells, in Shell Membranes, and in Meats of Clean, Dirty, and Washed-Dirty Shell Eggs

Sample	Clean	Dirty	Washed-dirty
Shells[1]	222.0	232.0	123.0
Number of shells positive for *Salmonella*	3.0	11.0	6.0
Per cent of shells positive for *Salmonella*	1.3	4.7	4.8
Ground shells[2]	87.0	89.0	54.0
Number of samples positive for *Salmonella*	2.0	14.0	7.0
Per cent of samples positive for *Salmonella*	2.3	15.7	12.9
Shell membranes[3]	37.0	33.0	39.0
Number of samples positive for *Salmonella*	0.0	3.0	6.0
Per cent of samples positive for *Salmonella*	0.0	9.0	15.4
Liquid egg, whole[4]	53.0	55.0	30.0
Number of samples positive for *Salmonella*	0.0	2.0	1.0
Per cent of samples positive for *Salmonella*	0.0	3.6	3.3
Yolk[5]	8.0	8.0	11.0
Number of samples positive for *Salmonella*	0.0	1.0[7]	0.0
Per cent of samples positive for *Salmonella*	0.0	12.5	0.0
White[6]	8.0	8.0	11.0
Number of samples positive for *Salmonella*	0.0	0.0	0.0
Per cent of samples positive for *Salmonella*	0.0	0.0	0.0

[1]The external shell surface of each intact egg was washed in 50 ml. Salenite-F. [2]A composite sample of two to four ground shells was introduced into 50 ml. Selenite-F. [3]Three to six membranes were introduced into 50 ml. Selenite-F. [4]Fifty ml. of a sample composited from three to six whole egg meats were inoculated into 50 ml. Selenite-F. [5]One to two yolks inoculated into 50 ml. Selenite-F. [6]Whites from one to two eggs inoculated into 50 ml. Selenite-F. [7]*S. pullorum* isolated from yolk of one egg and not found in any other portion of the egg.

SALMONELLA CONTAMINATION IN LIQUID WHOLE EGG PREPARED FROM CLEAN EGGS, "CANDLERS' CLEANS" LIGHT-DIRTY, AND WASHED-DIRTY EGGS

Source of Material and Method of Sampling: This study was conducted in two plants widely separated geographically. In Plant A (Texas), shell eggs designated as "clean" by the candlers and as "candlers' cleans" by the bacteriologists because of the presence of stained and slightly soiled (light-dirty) eggs, were resorted into clean and light-dirty eggs. In Plant M (Illinois), shell eggs taken directly from the receiving platform were candled and sorted by the bacteriologists into cleans, light-dirty, and dirty eggs. "Candlers' cleans" from the same carlots of eggs in this plant were also investigated. Washed-dirty eggs were examined in both Plants

A and M. The dirty eggs were hand washed with a cloth or brush after soaking for 10 to 20 minutes in a 100 to 200 p.p.m. hypochlorite solution (according to the method followed in the respective plants at the time of the study), then rinsed in cold tap water and drained.

For sampling material, eight to 10 dozen shell eggs of a selected class were broken out and dumped into a breaking pail. The contents of the pail were mixed and a 50-ml. sample was transferred directly to 50 ml. of Selenite-F.

TABLE 2

Salmonella Contamination in Liquid Whole Egg Prepared From Clean Eggs, "Candlers' Cleans," Light-Dirty, and Washed-Dirty Eggs

Sample	Dehydration plant	
	A	M
L-WE[1] from clean shell eggs resorted from plant-run "candlers' cleans"		
Number of samples examined	35.0	59.0
Number positive for *Salmonella*	1.0	3.0
Per cent positive for *Salmonella*	2.8	5.0
L-WE[1] from plant-run "candlers' cleans"[2]		
Number of samples examined	.....	64.0
Number positive for *Salmonella*	.....	11.0
Per cent positive for *Salmonella*	.....	17.1
L-WE[1] from light-dirty[3] shell eggs resorted from plant-run "candlers' cleans"		
Number of samples examined	35.0	67.0
Number positive for *Salmonella*	3.0	10.0
Per cent positive for *Salmonella*	8.8	15.0
L-WE[1] from washed-dirty shell eggs		
Number of samples examined	35.0	53.0
Number positive for *Salmonella*	5.0	11.0
Per cent positive for *Salmonella*	14 3	20.7

[1]L-WE=liquid whole egg; each sample represents the thoroughly mixed meats of eight to ten dozen shell eggs. [2]"Candlers' cleans" were sorted and classed as clean in the candling room; they usually contained stained and slightly soiled eggs (light-dirty) as well as the clean eggs. [3]Light-dirty were slightly soiled and slightly stained eggs only.

Results: The data are shown (Table 2). In Plant A, 105 buckets of liquid whole egg were broken out from candled eggs sorted according to degree of cleanliness. *Salmonella* organisms were recovered from one (three per cent) of 35 samples from cleans; from three (nine per cent) of 35 samples from light-dirty eggs; and from five (14 per cent) of 35 samples from washed-dirties. "Candlers' cleans" from the same carlots of shell eggs from which the other classes were obtained were not examined in Plant A, but it may be seen from Table 3 that 11 per cent of the "candlers' cleans" from other carlots were positive for *Salmonella.*

The results obtained at Plant M indicate a similar relationship. Three (five per cent) of 59 samples of liquid whole egg prepared from cleans were positive for *Salmonella,* 10 (15 per cent) of 67 samples from light-dirty shell eggs were positive, 11 (17 per cent) of 64 samples from "candlers' cleans" were positive, and 11 (21 per cent) of 53 samples from washed-dirty shell eggs were positive.

TABLE 3

Salmonella Contamination in Liquid Whole Egg at Different Processing Stages and in Spray-Dried Powder

Sample	Dehydration plant						Total
	A	B	C	R	H	M	
L-WE[1] from plant-run "candlers' cleans" shell eggs							
Number of samples examined	28	37	24	13	2	390	494.0
Number positive for *Salmonella*	3	4	2	1	0	43	53.0
Per cent positive for *Salmonella*	.....	.....	.....	.....	.....	.....	10.7
L-WE[1] from washed-dirty shell eggs							
Number of samples examined	36	34	23	13	2	.....	108.0
Number positive for *Salmonella*	9	6	5	2	0	.....	22.0
Per cent positive for *Salmonella*	.....	.....	.....	.....	.....	.....	20.4
L-WE[1] from unrefrigerated tanks (churns, mixing tanks, etc.)							
Number of samples examined	17	22	20	3	1	144	207.0
Number positive for *Salmonella*	3	8	7	2	0	33	53.0
Per cent positive for *Salmonella*	.....	.....	.....	.....	.....	.....	25.6
L-WE[1] from refrigerated cooling and holding tanks.							
Number of samples examined	16	12	24	6	1	70	129.0
Number positive for *Salmonella*	6	4	13	1	0	18	42.0
Per cent positive for *Salmonella*	.....	.....	.....	.....	.....	.....	32.6
D-WE[2] regular run-sifted							
Number of samples examined	19	18	12	4	1	46	100.0
Number positive for *Salmonella*	9	8	4	1	1	8	31.0
Per cent positive for *Salmonella*	.....	.....	.....	.....	.....	.....	31.0

[1]L-WE=liquid whole egg; each sample represents the thoroughly mixed meats of eight to ten dozen shell eggs. [2]D-WE=dried whole egg.

SALMONELLA CONTAMINATION IN LIQUID WHOLE EGG AT DIFFERENT PROCESSING STAGES AND IN SPRAY-DRIED POWDER

Source of Material and Method of Sampling: Samples for this study were obtained in six dehydration plants. In Plant M they were collected regularly during two and one-half months. Samples of liquid broken out from "candlers' cleans" and washed-dirties were obtained from the breaking-room buckets. Samples were also collected from churns (mixing or first holding tank) and from refrigerated tanks and vats. Fifty-ml. portions of each liquid sample were taken for examination and immediately inoculated into 50 ml. of Selenite-F. Samples of powder for analysis were composited from portions intermittently collected while it was being dispensed or were obtained with a sampling tube from the filled 14- or 100-pound containers. Eleven grams of powder was used for analysis and inoculated into 99 ml. of Selenite-F. For examination of a daily run, one sample was taken from each of five to 10 buckets of liquid broken out from "candlers' cleans," from five to 10 buckets broken out from washed dirties (when available), and at least two samples were taken from each of the churns and refrigerated tanks. One or two samples of powder were taken. Sampling throughout processing was progressive and at calculated intervals to permit following the same batch of liquid through the system.

Results: As taken in the breaking room during plant operation, 11 per cent of the samples of liquid whole egg prepared from regular plant run "candlers' cleans" contained *Salmonella* organisms (Table 3); 20 per cent of the samples prepared from washed-dirties were also positive. These findings substantiate the evidence obtained in the previous study with respect to the incidence of *Salmonella* contamination in liquid egg broken out from "candlers' cleans" and washed-dirties. As sampling progressed from the first receiving tank (usually a mixing tank or churn) to the refrigerated holding tank and the drier, increasingly greater percentages of positive samples were obtained. Twenty-five per cent of the samples of liquid from the first receiving tank, 33 per cent of the samples from the refrigerated holding tanks, and 31 per cent of the powder samples contained *Salmonella.*

SALMONELLA CONTAMINATION IN AND ON DUCK, GUINEA-HEN, TURKEY, AND GOOSE EGGS

Duck, guinea-hen, turkey, and goose shell eggs were collected and examined for the presence of *Salmonella* in essentially the same manner as the hen's eggs. Although a comparatively small number of these eggs were received at the plants, mixed in with the hen's eggs, it may be seen (Table 4) that *Salmonella* organisms were recovered from the shell surfaces of four of the 85 duck eggs and one of the 16 guinea-hen eggs examined, and also from one of the shell-membrane samples and from one of the ground-shell samples composited from turkey eggs. The same *Salmonella* type was isolated from the turkey-egg samples, both of which were composited from the same eggs. *Salmonella* organisms were not recovered from any of the goose-egg samples, or from the egg meats of any of the four different types of eggs.

DISTRIBUTION OF *SALMONELLA* TYPES ISOLATED DURING FIELD STUDIES FROM SHELL EGGS, LIQUID WHOLE EGG, AND SPRAY-DRIED WHOLE EGG

The variety and distribution of serological types of *Salmonella* recovered from shell eggs, liquid whole egg, and spray-dried whole egg are shown (Table 5). Eighteen types were recovered, 16 of which were recovered from Texas samples in February and March. Ten of the 16 types were also recovered from Illinois samples in August, September, and October, and, in addition, there were two types which had not previously been isolated during the Texas study. The geographical location of the dehydration plants or the season did not appear to be of any significance with respect to the types recovered insofar as could be determined from this limited study.

TABLE 4

Salmonella Contamination in and on Duck, Guinea-Hen, Turkey, and Goose Shell Eggs

Samples examined	Duck	Guinea-hen	Turkey	Goose
Shells (individual)	85	16	18	....
Positive for *Salmonella*	4	1	0	....
Ground-shell samples[1]	33	8	5	15[2]
Positive for *Salmonella*	2	0	1	0
Shell-membrane samples[1]	17	8	4	8
Positive for *Salmonella*	1	0	1	0
Whole egg-meat samples[1]	22	8	7	8
Positive for *Salmonella*	0	0	0	0

[1]Each sample composited from two to four eggs. [2]Each sample consisted of a single egg because of its size.

S. oranienburg was isolated with greatest frequency, followed in descending order by *S. pullorum* and *S. tennessee*. These three types accounted for 71 per cent of the isolations, the others being isolated sporadically and with diminishing frequency. The possibility of obtaining more than one type per sample except in rare instances was limited by the volume of field work, which seldom permitted more than two colony picks from any one sample. In 13 cases, however, two types were obtained from the same sample. Although the more common types were recovered from the samples collected during the same time interval, there was no evidence to indicate that samples collected during one of successive runs were necessarily contaminated with the same type of organism.

Nine of the 18 types isolated in the over-all study were recovered from the shell and ground-shell samples. In order of frequency of occurrence they were as follows: *S. oranienburg, S. montevideo, S. tennessee, S. bareilly, S. typhimurium, S. derby, S. essen, S. worthington, S. pullorum.* The significance of this finding cannot be properly evaluated since few shells were examined in proportion to the quantities of melange.

DISCUSSION

The evidence obtained in these studies indicates that the external shell surface is an important source of *Salmonella* contamination and that dirty

eggs are the primary offenders. These limited results do not, however, rule out the possibility of an internal yolk infection or the possibility that organisms in an infected yolk may migrate into the membrane and possibly into the shell. As shown (Tables 1 and 2) light-dirty eggs are much more likely to harbor *Salmonella* organisms than are clean ones. It is apparent that even slightly soiled eggs should not be classed by the candlers as "cleans." It is also apparent that washing dirty eggs by first soaking in a hypochlorite solution, followed by rubbing or brushing, rinsing in tap water, and draining is not a satisfactory process for removing undesirable microorganisms from shell surfaces. Soaking increases the probability of shell penetration, and subsequent rubbing or brushing may force the microorganisms into or through the shell pores.

TABLE 5

Distribution of Salmonella Types Isolated During Field Studies From Individual Shell Eggs, Liquid and Spray-Dried Whole Egg

Salmonella types	Recovered from		Isolations	
	Plants A, B, C, R, H	Plant M	Number	Pct. of total
S. oranienburg	44	73	117	42.2
S. pullorum	21	28	49	17.7
S. tennessee	12	19	31	11.2
S. montevideo	14	9	23	8.3
S. bareilly	6	7	13	4.7
S. meleagridis	0	7	7	2.5
S. derby	4	1	5	1.8
S. typhimurium	2	3	5	1.8
S. kentucky	0	4	4	1.4
S. essen	4	0	4	1.4
S. anatum	2	2	4	1.4
S. give	1	2	3	1.1
S. worthington	1	2	3	1.1
S. senftenberg	2	0	2	0.7
S. oslo	2	0	2	0.7
S. cerro	2	0	2	0.7
S. typhimurium var. Copenhagen	2	0	2	0.7
S. minnesota	1	0	1	0.4
Total	....	....	277	....

The finding of a wide variety of *Salmonella* types on shell surfaces and in the ground shell adds support to the evidence that contaminated eggshell surface is a significant source of the *Salmonella* organisms found in the finished powder. The fact that *S. pullorum* was not found with greater frequency may be attributed, in part at least, to the endeavor to simplify isolation technics in the early phases of the field work. No importance can be attached to the part contaminated duck, guinea-hen, turkey, and goose eggs may play in liquid egg or in powder contamination, because the use of these eggs in the manufacture of spray-dried whole-egg powder for sale to the Government is forbidden. The limited data on contamination of these types of eggs (Table 4) do not indicate that their infrequent use is of any major significance.

As to whether a continuous initial inoculum of *Salmonella* organisms in five per cent individual dirties (Table 1) can build up during processing or is distributed to the extent that 31 per cent of the dried whole egg (Table 3) is contaminated is at present only an interesting matter for conjecture. No especially satisfactory quantitative method is available for determining the number of *Salmonella* organisms in a given sample, particularly one which may be used conveniently in the field. The percentage increase in the incidence of contamination noted with progressive sampling during processing may be largely or even entirely accounted for by the aggregation of sampling material, by the more complete mixing and homogenization of the melange, and by the efficacy of the selective cultural methods used for isolation of these organisms. However, it was noted that in some plants, and on different occasions in the same plant, liquid egg was held unnecessarily long in unrefrigerated and in refrigerated tanks at high temperatures. Time and temperature relationships may at times be such as to enable microbial multiplication during processing. The fact that 31 per cent of the newly made powder samples were positive is conclusive evidence that the dehydration process as commonly practiced in the manufacture of powder of four to six per cent moisture content is not markedly destructive to *Salmonella* organisms.

From the standpoint of public health the importance of the isolation from any food product of a number of *Salmonella* types, most of which have been implicated in outbreaks of food poisoning, cannot be overestimated. The data obtained in these studies indicate that in order to reduce the incidence of *Salmonella* contamination of spray-dried whole-egg powder to a minimum, fresh, clean shell eggs should be used in its manufacture.

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NUTRITIVE VALUE OF CANNED FOODS. XVI. PROXIMATE AND MINERAL COMPOSITION[1]

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This work was undertaken as an integral part of the National Canners Association-Can Manufacturers Institute Nutrition program, in order to obtain a more thorough knowledge of the proximate and mineral composition of canned foods. A total of 1,384 samples, collected during the canning seasons of 1942 and 1943, were analyzed. The methods used by the nutrition committee in selecting the various foods, their geographical distribution, and methods of preparation of the 1942 samples have been described in detail by Clifcorn (1944). The methods of collecting samples during the 1943 season were similar to those of the previous year except that the following additional foods were included: blackberries, blueberries, cherries, cream style corn, kraut, mushrooms, peppers, pimentos, sweet potatoes, and turnip greens.

MATERIALS AND METHODS

The total contents[3] of four or six retail size cans or one to three No. 10 size cans comprising each sample were combined and thoroughly macerated in a Waring blendor. Portions of the uniformly blended mixture were then removed for the chemical analyses.

Chemical methods approved by the Association of Official Agricultural Chemists (1940) were used for all the determinations except iron, which was analyzed by Stugart's (1931) method modified as follows: (1) The colored iso-amyl alcohol layer was washed with 25 milliliters of a five-per cent solution of potassium sulfocyanide saturated with iso-amyl alcohol. (2) A per cent transmittance curve was prepared by plotting the per cent transmittance on a spectrophotometer at 490 millimicrons for a series of known solutions of pure iron. Per cent transmittance at 490 millimicrons was then obtained for each sample, and the amount of iron calculated by reference to the standard curve.

RESULTS

In a survey investigation of this nature, the average figures are of the greatest value since they indicate the average composition of the commodities as they are prepared under the variable conditions prevailing in different sections of the country and during different seasons and years; the results are summarized (Table 1).

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[2] With the technical assistance of Elizabeth Stephenson, Elden Floyd Johnson, and Mary Hoover Smith.

[3] Prunes were pitted before maceration.

TABLE 1

Proximate and Mineral Composition of Canned Foods[1]—(1942 and 1943 Samples)

Number of samples	Product	Grams per 100 grams						Milligrams per 100 grams		
		Moisture	Protein	Fat	Fiber	Ash	Carbohydrate	Calcium	Phosphorus	Iron
26	Apricots	77.91 ±1.17	0.53 ±0.09	0.19 ±0.07	0.41 ±0.04	0.53 ±0.17	20.45 ±1.19	8 ± 1	16 ± 1	0.7 ±0.5
16	Asparagus, bleached	93.34 ±0.42	1.61 ±0.23	0.33 ±0.08	0.54 ±0.06	1.46 ±0.19	2.71 ±1.08	15 ± 2	33 ± 4	0.9 ±0.7
40	Asparagus, green	93.55 ±0.54	1.86 ±0.33	0.28 ±0.06	0.51 ±0.07	1.26 ±0.25	2.53 ±0.41	18 ± 4	43 ± 6	1.7 ±1.5
5	Beans, baked	69.36 ±2.77	5.63 ±0.41	1.90 ±1.07	1.75 ±0.52	1.44 ±0.57	19.92 ±0.82	56 ± 6	106 ±23	2.6 ±1.3
112	Beans, green	93.47 ±0.80	1.05 ±0.21	0.14 ±0.07	0.65 ±0.15	1.23 ±0.18	3.45 ±0.73	34 ± 8	21 ± 9	1.2 ±0.8
53	Beans, lima	80.75 ±1.64	4.08 ±0.45	0.26 ±0.06	1.26 ±0.26	1.42 ±0.19	12.25 ±1.31	26 ± 4	67 ±11	2.4 ±0.5
47	Beets	90.29 ±1.06	0.92 ±0.24	0.09 ±0.05	0.52 ±0.06	0.80 ±0.21	7.38 ±1.06	14 ± 3	17 ± 6	1.8 ±1.5
6	Blackberries	76.03 ±2.65	0.74 ±0.09	0.16 ±0.02	2.93 ±0.34	0.30 ±0.04	19.84 ±2.74	18 ± 2	19 ± 3	2.6 ±1.6
6	Blueberries	88.54 ±1.44	0.56 ±0.08	0.18 ±0.04	0.40 ±0.05	0.16 ±0.02	10.17 ±1.57	11 ± 2	6 ± 2	5.1 ±1.5
39	Carrots	91.85 ±0.62	0.65 ±0.13	0.22 ±0.07	0.59 ±0.08	0.91 ±0.15	5.77 ±0.70	25 ± 3	20 ± 5	0.7 ±0.4
4	Cherries	86.57 ±0.57	0.81 ±0.20	0.27 ±0.15	0.12 ±0.04	0.45 ±0.08	11.79 ±0.28	11 ± 0	12 ± 0	2.8 ±0.4
11	Corn, white (CS)	76.51 ±2.00	2.17 ±0.14	0.61 ±0.13	0.57 ±0.12	1.04 ±0.12	19.10 ±1.92	2 ± 1	55 ± 9	0.6 ±0.1
76	Corn, white (WK)	81.80 ±1.75	1.90 ±0.31	0.56 ±0.12	0.63 ±0.14	0.84 ±0.15	14.26 ±1.58	5 ± 2	45 ± 6	0.5 ±0.2
25	Corn, yellow (CS)	76.16 ±2.12	2.12 ±0.19	0.63 ±0.16	0.50 ±0.12	1.06 ±0.17	19.52 ±1.98	3 ± 2	57 ± 9	0.6 ±0.1
114	Corn, yellow (WK)	80.37 ±1.41	1.93 ±0.26	0.60 ±0.11	0.52 ±0.17	0.96 ±0.18	15.58 ±1.58	4 ± 1	50 ± 7	0.4 ±0.2
6	Corn, yellow (WK), vacuum pack	75.52 ±1.03	2.47 ±0.18	0.54 ±0.05	0.82 ±0.09	1.05 ±0.07	19.58 ±0.91	3 ± 1	73 ± 3	0.5 ±0.1
54	Grapefruit juice	88.66 ±1.02	0.47 ±0.04	0.14 ±0.07	0.04 ±0.01	0.38 ±0.07	10.32 ±1.00	8 ± 2	13 ± 2	0.4 ±0.3
29	Grapefruit segments	80.22 ±1.69	0.57 ±0.07	0.14 ±0.10	0.23 ±0.08	0.38 ±0.04	18.45 ±1.57	13 ± 2	14 ± 3	0.3 ±0.1
6	Kraut	92.36 ±0.71	0.98 ±0.05	0.21 ±0.03	0.67 ±0.06	1.97 ±0.18	3.82 ±0.68	36 ± 5	18 ± 3	6.9 ±2.1
7	Mackerel	64.33 ±1.83	19.21 ±1.78	13.16 ±2.36	0.09 ±0.06	2.81 ±0.80	0.40 ±0.31	225 ±41	274 ±57	1.9 ±0.3
5	Mushrooms	93.19 ±0.90	2.89 ±0.14	0.13 ±0.02	0.66 ±0.07	1.63 ±0.09	1.50 ±0.52	6 ± 1	68 ± 5	0.5 ±0.2

7	Mackerel	64.33 ±1.83	19.21 ±1.78	13.16 ±2.36	0.09 ±0.06	2.81 ±0.80	0.40 ±0.31	225 ±41	274 ±57	1.9 ±0.3
5	Mushrooms	93.19 ±0.90	2.89 ±0.14	0.13 ±0.02	0.66 ±0.07	1.63 ±0.09	1.50 ±0.52	6 ± 1	68 ± 5	0.5 ±0.2
36	Orange juice	87.60 ±1.07	0.79 ±0.20	0.18 ±0.06	0.03 ±0.01	0.46 ±0.04	10.94 ±1.15	10 ± 2	19 ± 3	0.5 ±0.3
25	Peaches, cling	79.41 ±2.61	0.45 ±0.07	0.13 ±0.07	0.28 ±0.03	0.32 ±0.06	19.41 ±2.67	3 ± 1	11 ± 2	0.3 ±0.2
20	Peaches, freestone	77.93 ±3.69	0.45 ±0.10	0.08 ±0.02	0.35 ±0.06	0.33 ±0.08	20.83 ±3.66	4 ± 1	12 ± 2	0.3 ±0.2
44	Pears	80.65 ±1.90	0.25 ±0.06	0.11 ±0.05	0.69 ±0.22	0.20 ±0.04	18.09 ±1.84	6 ± 1	7 ± 2	0.5 ±0.2
37	Peas, Alaska	82.59 ±1.86	3.46 ±0.53	0.25 ±0.07	1.46 ±0.19	1.14 ±0.15	11.11 ±1.49	20 ± 3	66 ±11	1.7 ±0.5
150	Peas, sweet	84.75 ±1.69	3.43 ±0.47	0.35 ±0.12	1.41 ±0.18	1.07 ±0.16	8.98 ±1.29	19 ± 4	58 ±12	1.5 ±0.6
2	Peppers	91.05 ±0.0	0.83 ±0.0	0.37 ±0.0	0.63 ±0.0	1.11 ±0.0	6.02 ±0.0	8 ± 0	19 ± 0	1.8 ±0.0
3	Pimentos	93.45 ±0.0	0.87 ±0.0	0.38 ±0.0	0.68 ±0.0	0.36 ±0.0	4.27 ±0.0	6 ± 0	15 ± 0	1.3 ±0.0
17	Pineapple juice	84.87 ±0.52	0.40 ±0.05	0.10 ±0.09	0.08 ±0.04	0.37 ±.04	14.18 ±0.50	15 ± 3	9 ± 2	0.3 ±0.2
17	Pineapple slices	74.04 ±0.89	0.37 ±0.05	0.18 ±0.12	0.31 ±0.03	0.37 ±0.11	24.73 ±0.95	20 ± 9	8 ± 2	0.7 ±0.5
5	Potatoes, sweet	70.88 ±1.60	1.46 ±0.31	0.06 ±0.04	1.04 ±0.10	0.87 ±0.07	25.69 ±1.56	21 ± 5	50 ± 8	0.6 ±0.1
15	Prunes (edible portion only)	76.11 ±1.41	0.45 ±0.05	0.14 ±0.05	0.24 ±0.02	0.48 ±0.09	22.58 ±1.37	8 ± 1	13 ± 1	1.0 ±0.5
5	Salmon	68.74 ±2.84	21.43 ±0.30	6.97 ±2.10	0.05 ±0.0	2.69 ±0.20	0.12 ±0.0	184 ±33	292 ±21	0.6 ±0.1
9	Sardines in oil	54.39 ±4.35	19.55 ±1.62	21.74 ±4.68	0.12 ±0.0	3.79 ±0.46	0.49 ±0.0	354 ±56	434 ±42	3.5 ±0.6
16	Sardines in tomato sauce	63.11 ±3.13	17.77 ±0.73	14.78 ±3.49	0.20 ±0.03	2.68 ±0.28	1.46 ±0.61	381 ±46	168 ±28	4.1 ±1.5
3	Shrimp, dry pack	66.45 ±2.80	26.73 ±1.73	1.26 ±0.15	0.11 ±0.06	5.04 ±1.33	±0.0 0.41	104 ±21	240 ±25	3.5 ±1.1
5	Shrimp, regular pack	78.59 ±0.88	14.97 ±0.64	0.73 ±0.04	0.07 ±0.04	5.20 ±0.84	0.44 ±0.0	55 ± 8	149 ±10	2.2 ±0.4
55	Spinach	92.96 ±0.88	2.04 ±0.42	0.42 ±0.08	0.68 ±0.08	1.64 ±0.23	2.26 ±0.59	85 ±23	26 ± 8	2.1 ±0.9
84	Tomatoes	93.75 ±0.67	0.97 ±0.13	0.15 ±0.09	0.41 ±0.09	0.75 ±0.16	3.97 ±0.68	6[2] ± 1	19 ± 4	0.5 ±0.3
138	Tomato juice	93.66 ±0.79	0.84 ±0.14	0.13 ±0.07	0.17 ±0.04	1.14 ±0.15	4.05 ±0.74	7 ± 2	18 ± 3	0.9 ±0.7
6	Turnip greens	93.71 ±0.96	1.51 ±0.31	0.33 ±0.08	0.66 ±0.08	1.29 ±0.23	2.50 ±0.64	100 ±24	30 ± 7	4.6 ±1.5
6	Tuna fish	55.42 ±2.84	23.99 ±1.79	17.34 ±3.43	0.25 ±0.05	2.10 ±0.53	0.90 ±0.50	8 ± 0	224 ±14	1.2 ±0.5

[1] Total can contents given on wet-weight basis. [2] No calcium added; 28 samples to which calcium was added = 28 ± 2.

The accompanying standard deviation measures in each case the dispersion of results about the average. Since this statistic is based upon all the results, it is a far more efficient measure of variation than the range between maximum and minimum, either of which may, and frequently does, reflect some unusual condition. The standard deviation also serves as a basis for estimating the interval within which any stated portion of the results may be expected to fall. For example, in a normal distribution, a range of one standard deviation each side of the average includes 68 per cent of the results, and a range of 1.65 times the standard deviation each way includes 90 per cent.

Finally, the standard deviation may be used in statistical comparisons of the composition of different products. Given the standard deviation and the number of samples, it becomes possible to determine by suitable statistical calculation whether the differences between averages are actually significant or merely due to chance alone.

A regrouping of canned foods in descending order of importance, as a source of each of the nutritive values is presented (Table 2). Those commodities which had the highest average level for each nutritive value and whose ranges in variability overlapped that of the highest commodity as measured by the standard deviation were placed in the first class. The other groupings were accomplished in a similar manner using the next highest average levels and deviations.

VARIATIONS AMONG COMMODITIES

Protein: As might be expected, the fish products are high in protein. The legumes, baked beans, lima beans, and peas, are in the next two groups followed by the other vegetables. Fruits, which are poor sources of protein, are found in the last two classes.

Fat: The fish products are also high in fat content, while only a few of the canned vegetables and none of the canned fruits analyzed have significant quantities of oil.

Carbohydrates: Sweet potatoes, sweet corn, and most of the fruits are excellent sources of carbohydrates. The fish products are the lowest in carbohydrate content.

It should be pointed out that the carbohydrate content of the vegetables consists mostly of starch and other polysaccharides stored by the plant organs, while the carbohydrate content of the fruits consists mostly of the syrup added to the fruit upon canning.

Calcium: Fish which are canned whole are of course high in calcium, but boned fish are relatively low in calcium content. The leafy vegetables, spinach and turnip greens, are fairly high in calcium content. Most of the other vegetables and fruits contain only a small amount of calcium.

Phosphorus: The fish products are very high in phosphorus followed by the seed vegetables which will average about 25 per cent of the phosphorus content of fish. Most of the other vegetables and fruits contain about half the phosphorus content of the seed vegetables.

Iron: The iron content of canned food depends more on the effect of the food upon the iron in the container walls than upon the original amount of iron in the raw product. Thus, for example, canned blueberries contain

TABLE 2

Classification of Canned Foods for Proximate and Mineral Components

Protein (approximate)	Products
pct.	
27	Shrimp, dry pack; tuna fish
21	Salmon; sardines in oil; mackerel
18	Sardines in tomato sauce
15	Shrimp, regular pack
6	Beans, baked
4	Beans, lima; peas
3	Mushrooms
2.5	Corn, vacuum pack; corn, cream style; spinach
2	Corn, whole kernel; asparagus; sweet potatoes; turnip greens
1	Beans, green; tomatoes; tomato juice; pimentos; peppers; orange juice; kraut; cherries; beets
0.7	Blackberries; carrots; apricots; blueberries; grapefruit; grapefruit juice; peaches
0.5 or less	Prunes; pears; pineapple juice; pineapple segments

Fat (approximate)	Products
pct.	
20	Sardines; tuna fish
12	Mackerel
7	Salmon
2	Beans, baked; shrimp, dry pack
0.7	Shrimp, regular pack; corn, cream style; corn, yellow, whole kernel
0.5	Corn, white, whole kernel; peas, sweet; peppers; pimentos; spinach
0.3 or less	Apricots; asparagus; beans, green; beans, lima; blackberries; blueberries; carrots; cherries; grapefruit juice; grapefruit segments; kraut; mushrooms; orange juice; peaches; peas, Alaska; pineapple; pineapple juice; prunes; tomatoes; tomato juice; turnip greens; beets; pears; sweet potatoes

Carbohydrates (approximate)	Products
pct.	
25	Sweet potatoes; pineapple slices; peaches, freestone
22	Apricots; corn, yellow, cream style; peaches, cling; prunes; blackberries
19	Beans, baked; corn, white, cream style; corn, whole kernel; grapefruit segments; pears
14	Corn, white, whole kernel; pineapple juice
12	Beans, lima; blueberries; cherries; grapefruit juice; orange juice; peas, Alaska
9	Beets; peas, sweet; peppers; carrots
4	Asparagus, bleached; beans, green; kraut; pimentos; tomatoes; tomato juice
2	Asparagus, green; mushrooms; sardines in tomato sauce; spinach; turnip greens
1 or less	Mackerel; salmon; sardines in oil; shrimp; tuna fish

Calcium (approximate)	Products
mg. pct.	
350	Sardines
200	Mackerel, salmon
100	Shrimp, dry pack; spinach; turnip greens
50	Beans, baked; shrimp, regular pack

TABLE 2 *(Concluded)*

(approximate) Calcium	Products
mg. pct.	
40	Beans, green; beans, lima; carrots; kraut; pineapple slices; sweet potatoes
20	Asparagus; beets; blackberries; grapefruit segments; peas; pineapple juice
10	Apricots; blueberries; cherries; grapefruit juice; orange juice; tomato juice; peppers; prunes; tuna fish
6	Corn, whole kernel; corn, yellow, cream style; mushrooms; peaches, freestone; pears; pimentos; tomatoes
4 or less	Corn, white, cream style; peaches, cling
Phosphorus (approximate)	**Products**
mg. pct.	
400	Sardines in oil
300	Salmon, mackerel
225	Shrimp, dry pack; tuna fish
175	Sardines in tomato sauce; shrimp, regular pack
100	Beans, baked
65	Beans, lima; corn, cream style; corn, vacuum pack; corn, yellow, whole kernel; mushrooms; peas; sweet potatoes
45	Corn, white, whole kernel; asparagus, green
30	Asparagus, bleached; beans, green; beets; carrots; peppers; spinach; tomatoes; turnip greens
30	Apricots; blackberries; grapefruit segments; kraut; orange juice; pimentos; tomato juice
13	Cherries; grapefruit juice; peaches; pineapple juice; prunes
10 or less	Blueberries; pears; pineapple slices
Iron (approximate)	**Products**
mg. pct.	
6.0	Blueberries; kraut; sardines in tomato sauce; turnip greens
3.0	Asparagus, green; beans, baked; beans, lima; beets; cherries; blackberries; sardines in oil; shrimp; spinach
1.5	Apricots; asparagus, bleached; beans, green; mackerel; peas; peppers; pimentos; pineapple slices; prunes; tomato juice; tuna fish
0.7 or less	Carrots; corn; grapefruit juice; grapefruit segments; mushrooms; orange juice; peaches; pears; pineapple juice; sweet potatoes; salmon; tomatoes

more iron than canned peas, although the reverse is true with the raw products.

VARIATIONS WITHIN THE COMMODITY

There is a great variation among samples of the same commodity as is clearly shown (Table 1). This was also true of the vitamin values of the 1942 samples [Pressley, Ridder, Smith, and Caldwell (1944); Ives, Wagner, Elvehjem, and Strong (1944); and Thompson, Cunningham, and Snell (1944)].

A complete explanation of the variability found would call for a study of factors beyond those considered here. Some of the factors causing this variability, however, become apparent from a study of the individual sample analyses. Because of consideration of space these individual sample data could not be presented.

Moisture or Total Solids: The variation in solids among samples of fruits may be due largely to the density of the syrup added to the canned product. The variation among the starchy vegetables may be attributed to differences in the maturity of the raw product before processing. The controlling factor which seems to affect solids in tomatoes and tomato juice is the availability of water to the growing plants, Hamner and Maynard (1942). In sweet potatoes, corn, shrimp, etc., the style of pack may affect total solids. Sweet potatoes packed solidly in the can would obviously contain more solids than whole sweet potatoes packed in syrup.

Protein: Protein content of fruits is generally very low and therefore relatively unimportant from a nutritional standpoint. Protein content of canned vegetables appears to be positively correlated with total solids. The starchy vegetables generally have a higher protein content when the raw material is more mature.

Fat: The fat content of most fruits and vegetables is so low as to be nutritionally insignificant. Variations in fat content of baked beans may be attributed largely to differences in the style of pack. Variations in fat content of sardines are of course largely due to the amount of oil added or, in the case of those styles where packing media other than oil are used, due to the variable efficiency of the individual plant practice in removal of portions of the natural oil content of the fish during canning.

Fiber: Fiber content of starchy vegetables is also positively correlated with total solids. The large variability in fiber content among pear samples is due presumably to varietal differences as well as to the degree of coring.

Carbohydrates: In general, carbohydrates are closely correlated to total solids. It is interesting to note that in the case of tomatoes and tomato juice a limitation of available moisture tends to increase the carbohydrate content but has little effect on the protein or other proximate components. The composition of beets is affected in a similar manner.

Calcium: Asparagus spears appear to be higher in calcium than cuts. Calcium contents of tomatoes fall into two distinct groups, one averaging about five mg. per cent, the other about 28 mg. per cent. It is presumed that samples in the latter group were packed with the addition of calcium chloride as permitted in the Federal standard of identity, under which the maximum added calcium chloride corresponds to about 25 mg. per cent of calcium. There is a remarkably close negative correlation between the calcium and total solids content of corn, thus indicating that the calcium content of corn decreases with increasing maturity. This appears to be true of other seed products, though not in such a definite manner. The calcium content of canned mackerel was found to average 225 mg. per cent in contrast to 11 mg. per cent [American Can Company (1943)] and five mg. per cent [Nilson and Coulson (1939)] reported for the raw product. This disagreement is obviously due to the fact that the bones were included in the analyses of the canned product.

Phosphorus: Phosphorus content of seed vegetables is generally high. In contrast to calcium content, phosphorus tends to increase with maturity. This is particularly true for green beans where the seed content increases rapidly with maturity.

Iron: The relatively great variability in iron content is due largely to the amount of iron that is removed from the walls of the container. In most products there is a close relationship between iron content and length of storage of the product in the can. Thus when the can is freshly packed, most of the iron recovered from the product is provided by the food itself. Upon standing, additional iron enters the food from the container walls.

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BACTERIOLOGICAL STUDIES RELATING TO THERMAL PROCESSING OF CANNED MEATS

IV. VIABILITY OF SPORES OF A PUTREFACTIVE ANAEROBIC BACTERIUM IN CANNED MEAT AFTER PROLONGED INCUBATION

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The influence of curing agents upon the thermal resistance of a putrefactive anaerobic bacterium in canned meats was reported by Stumbo, Gross, and Vinton (1945b). Duplicate tubes from this study were held under incubation at 28°C.(82.4°F.) for one year and were examined at intervals for visual evidence of growth. The results of this study were reported by Stumbo, Gross, and Vinton (1945c). Inasmuch as there appeared to be inhibition of growth in some cases it would be interesting to determine whether or not viable spores were present in those tubes not showing visual evidence of growth but in which there had been viable spores at the start of the incubation period.

EXPERIMENTAL PROCEDURE

All tubes, whether showing visual evidence of growth or not, were cultured into dextrose brain broth using the technique outlined by Stumbo, Gross, and Vinton (1945a). The culture tubes were incubated for 14 days at 37°C.(98.6°F.). The data for the inoculation of 10 and 10,000 spores per gram are reported (Tables 1 and 2) and for the sake of comparison the data reported in the earlier work are also included. The data for the control, 5-, 100-, 500-, and 1,000-spore inoculations are omitted in the interest of simplicity and because they showed no significant variations from the data reported.

DISCUSSION

Stumbo, Gross, and Vinton (1945c) discussed the inhibiting effect as shown in this data by the comparison of the visual evidence of growth in the tubes with the presence of viable spores in a duplicate set of tubes cultured immediately after processing. Definite inhibition was noted and the conclusions were in agreement with those made by Tanner and Evans (1933a, b and 1934) and Evans and Tanner (1934) in their investigations on the effect of meat-curing solutions on anaerobic bacteria. Yesair and Cameron (1942) made similar observations but attributed some specificity of action to salt, nitrate, and nitrite while Tanner and Evans and the authors did not. Yesair and Cameron used a different technique for evaluating the inhibitive effect by counting the reduction in colonies compared with a control, whereas the authors had used the end-point technique which does not permit drawing such fine distinctions. The effect of nitrate and nitrite is still a controversial subject. Jensen (1942) reviewed the subject

and presented an especially good historical background. The comments of Tanner (1944) should also be consulted for still further discussion on this subject.

In the opinion of the authors the fact that sodium nitrate is a constituent to the extent of 0.2 per cent in media recommended for the isolation and identification of anaerobes by Spray (1936) cannot be overlooked. This is a slightly larger amount than the maximum (2¾ ounces per hundred weight of comminuted meat corresponding to 0.17 per cent sodium nitrate) permitted for use in federally inspected packing houses. Further, the data reported by Moulton and Lewis (1940) show that proteolysis as measured by Sorenson number is actually stimulated by sodium nitrate and sodium nitrite in the ranges of 0.1 to 0.2 and 0.01 to 0.02 per cent, respectively. These values are within the upper limits permitted for use in federally inspected packing houses. Further, it is the opinion of the authors that such percentages of nitrate and nitrite are too low to be of major significance in affecting the thermal processing levels required for satisfactory commercial operation either through lowering the thermal resistance of the spores or inhibiting the germination of viable spores. Salt is an ingredient of major significance with regard to inhibition. Nitrate is relatively inert and nitrite, while it may have an effect when present as nitrite ion, is so labile chemically that it is usually present only in minute amounts in the finished canned meat product. The foregoing opinions are based upon evidence obtained through study of the action of these curing agents on putrefactive anaerobe S_2 in canned meats. It is possible and perhaps even probable that different evidence might be obtained with other organisms. However, it appears that the spores of S_2 would be about the most heat resistant of any potential spoilage organism that might normally be expected to be encountered in the thermal processing of canned meats. Canned-meat products containing other than meat and curing agents would be an exception to this statement and the picture would then be complicated by the possible organisms contained in the added substances other than meat.

Yesair and Cameron (1942) state that in the earlier work they found the end point of spoilage in inoculated-pack studies was not the end point of destruction and that they were able to isolate the test organisms from unspoiled cans which had been processed above the spoilage level. Similar data were obtained in this investigation which proves that there was an inhibition of germination of spores. Although the spores did not germinate in the test substrate after an incubation period of one year at 28°C. (82.4°F.) they did germinate within a normal period of from 48 to 120 hours at 37°C.(98.6°F.) when cultured into another media which by dilution reduced the concentration of the inhibiting factors. The data show that viable organisms were found in tubes receiving as much as eight F_o values greater heat process than the process given the last tube to show visual evidence of spoilage. This is an important observation in connection with commercial canning practice.

The effectiveness of salt is demonstrated by the greatly reduced inhibition of 1½ per cent salt compared with 3½ per cent. Whether or not there is inhibition shown in the experiments on nitrate and nitrite is open

TABLE 1

Thermal Resistance, Visual Growth, and Dormancy Using an Inoculation of 10 Spores

Processed at 115.6°C.(240°F.)		Meat treated 24 hours prior to processing with—																																	
Time	F_o value	Nothing			Sugar			Sugar, nitrate			Sugar, nitrite			Sugar, nitrate, nitrite			Sugar, salt 3½			Sugar, salt 3½, nitrate			Sugar, salt 3½, nitrite			Sugar, salt 3½, nitrate, nitrite			Sugar, salt 1½, nitrate, nitrite					
		A	B	C[1]	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C			
min.																																			
5.77	1	++	aa	++	++	aa	++	++	aa	++	++	ee	++	++	eg	++	++	hi	++	++	eg	++	++	hh	++	++		++	++	eh	++				
9.37	2	++	aa	++	++	aa	++	++	aa	++	++	be	++	++	fi	++	++	i	++	++	ii	++	++	i	++	++	−−	++	++	gi	++				
12.96	3	++	ab	++	++	aa	++	++	ab	++	++	hh	++	++	fh	++	++		++	++	i	++	++		+−	++	+−	++	++	i	++				
16.56	4	+−	be	++	++	ab	++	++	bc	++	++	h	++	++	hh	++	++		++	++		−−	++		−−	++	−−	++	+−	i	++				
20.15	5	−−	be	++	++	bb	++	++	bd	++	++	hh	++	++		++	+−		+−	++		−−	+−		−−	+−	−−	−−		−−				
23.74	6	−−	h	+−	−−	bd	++	+−	ce	++	+−		++	++		++	+−		−−	++		−−	++		−−	−−	−−	−−		−−				
27.34	7	−−	h	++	−−		−−	++	dd	++	+−		−−	−−		−−	+−		−−	++		+−	+−		−−	+−	−−	−−		−−				
30.93	8	−−		−−	−−		−−	−−	e	+−	−−		−−	−−		−−	−−		−−	++		−−	−−		−−	−−	−−	−−		−−				
34.53	9	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−	−−	−−		−−				
38.12	10	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	+−		−−	−−		−−	−−		−−	−−	−−	−−		−−				
41.71	11	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−	−−	−−		−−				
45.31	12	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−	−−	−−		−−				
48.90	13	−−			−−			−−			−−			−−			−−			−−			−−			−−			−−						
52.50	14	−−			−−			−−			−−			−−			−−			−−			−−			−−			−−						
56.09	15	−−			−−			−−			−−			−−			−−			−−			−−			−−			−−						
59.68	16	−−			−−			−−			−−			−−			−−			−−			−−			−−			−−						

[1]A—Tubes cultured immediately after heat processing [Stumbo, Gross, and Vinton (1945b)].
B—Spoilage by visual observation of unopened tubes incubated one year at 28°C.(82.4°F.). [Stumbo, Gross, and Vinton (1945c)].
C—Tubes cultured after one year of incubation at 28°C.

Key: a..... 2 days
b..... 6 days
c.....11 days
d.....18 days
e.....32 days
f.....40 days
g..... 54 days
h..... 77 days
i.....111 days
j.....249 days
k.....365 days

TABLE 2

Thermal Resistance, Visual Growth, and Dormancy Using an Inoculation of 10,000 Spores

Processed at 115.6°C.(240°F.)		Meat treated 24 hours prior to processing with—																														
Time	F_o value	Nothing			Sugar			Sugar, nitrate			Sugar, nitrite			Sugar, nitrate, nitrite			Sugar, salt 3½			Sugar, salt 3½, nitrate			Sugar, salt 3½, nitrite			Sugar, salt 3½, nitrate, nitrite			Sugar, salt 1½, nitrate, nitrite		
		A	B	C[1]	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
min.																																
5.77	1	++	aa	++	++	aa	++	++	aa	++	++	cc	++	++	bb	++	++	eg	++	++	cc	++	++	hh	++	++	gg	++	++	bb	++
9.37	2	++	aa	++	++	aa	++	++	aa	++	++	bc	++	++	bb	++	++	gg	++	++	cc	++	++	hi	++	++	h	++	++	cd	++
12.96	3	++	aa	++	++	aa	++	++	aa	++	++	bb	++	++	bc	++	++	hi	++	++	cc	++	++		++	++		++	++	ee	++
16.56	4	++	aa	++	++	aa	++	++	aa	++	++	cc	++	++	bg	++	++	i	++	++		++	++		++	++		++	++	eg	++
20.15	5	++	aa	++	++	aa	++	++	aa	++	++	cd	++	++	bg	++	++		++	++		++	++		++	++		++	++	fg	++
23.74	6	++	aa	++	++	aa	++	++	aa	++	++	de	++	++	fg	++	++		++	++		++	++		+−	++		+−	++	gg	++
27.34	7	++	bb	++	++	ab	++	++	aa	++	++	ee	++	++	hh	++	++		++	++		++	+−		+−	++		+−	++	hh	++
30.93	8	++	ab	++	++	bb	++	++	bb	++	++	gg	++	++	gg	++	++		++	++		++	−−		−−	+−		++	++		+−
34.53	9	+−	ae	++	+−	bb	++	++	bc	++	+−	gg	++	+−	hh	++	++		+−	++		++	−−		−−	−−		+−	++		+−
38.12	10	−−	be	++	−−	bd	++	++	cc	++	−−	i	++	−−		−−	+−		−−	++		+−	−−		−−	−−		−−	+−		−−
41.78	11	−−		+−	−−	h	++	−−	dd	++	−−		−−	−−		−−	−−		−−	++		+−	−−		−−	−−		−−	−−	i	+−
45.31	12	−−		+−	−−		−−	−−		++	−−		−−	−−		−−	+−		−−	+−		+−	−−		−−	−−		−−	−−		−−
48.90	13	−−		+−	−−		+−	−−		++	−−		−−	−−		−−	−−		−−	++		+−	−−		−−	−−		−−	−−		−−
52.50	14	−−		+−	−−		−−	−−		++	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−
56.09	15	−−		+−	−−		−−	−−		++	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		−−
59.68	16	−−		−−	−−		−−	−−		++	−−			−−		−−	−−		−−	−−		−−	−−		−−	−−		−−	−−		

[1]A—Tubes cultured immediately after heat processing [Stumbo, Gross, and Vinton (1945b)].
B—Spoilage by visual observation of unopened tubes incubated one year at 28°C.(82.4°F.). [Stumbo, Gross, and Vinton (1945c)].
C—Tubes cultured after one year of incubation at 28°C.

Key: a..... 2 days
b..... 6 days
c.....11 days
d.....18 days
e.....32 days
f.....40 days
g..... 54 days
h..... 77 days
i.....111 days
j.....249 days
k.....365 days

to question and the authors believe that conclusions are not warranted because the reproducibility of the data is believed to involve about two F_0 units, so significant results should show a difference greater than two F_0 units. The data show that salt in a concentration of 3½ per cent is the major inhibiting ingredient. If the effect of nitrate and nitrite is positive, it is too small to be of great significance in commercial practice.

There are some discrepancies but when an agreement no better than two F_0 values is accepted as the possible error it can be seen that in general the various data agree well. Also the data are as free from "skips" as could be expected when using the end-point technique of thermal death-time evaluation. The data indicate that in general there is no loss of viability or destruction of spores during the incubation period even though they may not germinate. The exceptions to this (within the postulated experimental error of two F_0 values) (Table 1) are when 3½ per cent salt, alone or in combination with either or both nitrate and nitrite, is present when there appears to be an indication of destruction of spores at the higher processing levels during the incubation period. This does not appear to be true in the experiment using 1½ per cent salt. This effect is not clear-cut and this work should be checked before conclusions are drawn. An opposite effect noted (Table 1) on meat alone and (Table 2) on meat alone, meat with sugar and nitrate, and questionable on meat and sugar is that there appeared to be viable spores after incubating one year at processing levels beyond indicated destruction as determined when tubes were cultured immediately after processing. It is suggested that in these cases there are no secondary inhibitors present and possibly this condition is related to that known as dormancy. At least this suggestion is offered as a possible explanation although more work is needed before conclusions should be drawn.

It is interesting to note that with regard to dormancy of spores there appears to be considerable controversy. It is thought worth while to call attention to this subject because it may have an important bearing on the commercial aspects of canning practice. Topley and Wilson (1941) discuss this subject under the heading "Effect of Heat on Subsequent Multiplication." They review references regarding this controversial subject and conclude that the evidence appears to them to be more in favor of the interpretation that there has been an effect of the heat upon the spores to increase the lag period of growth of those remaining viable organisms in contrast to the explanation that the increased resistance is due to an impermeable cell membrane that also restricts the power of rapid reproduction. Tanner (1944) also reviews this subject and points out the possible commercial significance in canning technology. In the review by Tanner the evidence appears to be interpreted toward the impermeable cell membrane theory since unheated cells may also show dormancy. Dickson, Burke, Beck, and Johnston (1925) found that heated spores of *Cl. botulinum* may be delayed in germinating as much as 330 days. Therefore, it is not impossible that the authors may not have held the cultures sufficiently long to be certain that there would be no further germination although upon culturing into another media, growth was noted in the usual period of 48 to 120 hours at 37°C.(98.6°F.).

SUMMARY

The presence of viable spores was demonstrated in cultures of tubes showing no visual spoilage after incubation for one year at 28°C. (82.4°F.). This was explained as inhibition of germination of the viable spores. The effectiveness of inhibition increased with an increased percentage of salt but was not completely effective in any case. The effect of nitrate and nitrite could not be clearly demonstrated and if the effect were positive it would be regarded as negligible compared with that of 3½ per cent salt.

Dormancy of viable spores was evident especially at processing levels approaching destruction. Dormancy was observed even in the absence of secondary inhibitors, such as salt.

In all tubes containing salt the visual evidence of spoilage was noted only after several months' incubation. Viable spores were demonstrated at thermal-processing levels as much as eight F_0 units above those showing visual evidence of spoilage. These observations may be of considerable importance in their possible application to canning technology.

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BACTERIOLOGICAL STUDIES RELATING TO THERMAL PROCESSING OF CANNED MEATS

V. CHARACTERISTICS OF PUTREFACTIVE ANAEROBE USED IN THERMAL RESISTANCE STUDIES

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The test organism used in this series of investigations was isolated as the causative agent in the spoilage of canned meat. Culture S_2 was isolated in this laboratory and Culture 9x in the Research Laboratories of the American Can Company, Maywood, Illinois.

Several other cultures of putrefactive anaerobes were included in this study for comparative purposes. A culture of a test organism, P. A. 3679, used frequently in inoculated-pack studies was obtained from the Research Laboratories of the National Canners' Association. This organism was mentioned by Williams (1940) as *Clostridium sporogenes,* strain No. 3679, having a heat resistance somewhat in excess of the recorded maximum for *Clostridium botulinum.* In later references there is no mention of *Cl. sporogenes* and reference is only to P. A. 3679. Cameron[2] states that although it resembles *Cl. sporogenes* it is probably not identical with it. Sognefest and Benjamin (1944) also refer to it as *Cl. sporogenes.* It was included in this investigation because it has been referred to as *Cl. sporogenes,* it has commonly been used as an inoculated-pack test organism, and its spores are reported to have a high heat resistance. Two cultures of *Cl. sporogenes* were used. One, *Cl. sporogenes* (Spray), was obtained from the Research Laboratories of the American Can Company, Maywood, Illinois, and was originally obtained by them from Dr. R. S. Spray of the West Virginia Medical School; and the other *Cl. sporogenes* (J-1), was obtained from Dr. J. E. Stritar, Research Laboratories, American Meat Institute, Chicago, Illinois. A study was made of the fermentation reactions of these five putrefactive anaerobes. Serological studies were made on the relationship of P. A. 3679, *Cl. sporogenes* (Spray), S_2, and 9x.

FERMENTATION REACTIONS

An attempt was made to determine fermentation reactions by observation of color change in a semisolid medium, Spray (1936), containing brom cresol purple indicator, but this procedure was difficult and possibly unreliable because of the destruction of the indicator within 48 hours. A second experiment was conducted using the same medium without indicator and determining the pH of the medium after six days' growth of the organism.

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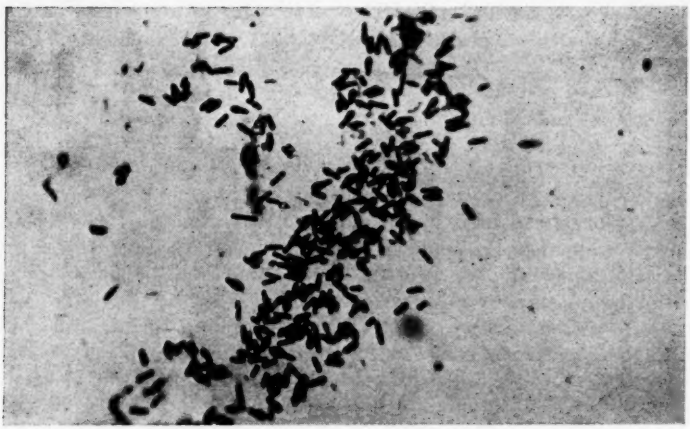

FIG. 1. Photomicrograph showing spore formation. Carbol fuchsin stain prepared from deep shake North's gelatin agar culture at 14 days.

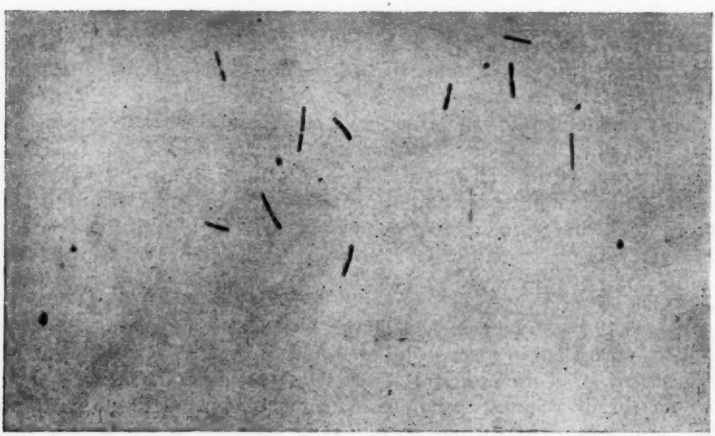

FIG. 2. Photomicrograph showing vegetative cells with tendency toward chain formation. Gram stain prepared from glucose brain broth culture at 48 hours.

The fermentation of starch and dextrin by three, inulin by one, and salicin by two of the organisms was detected by this method and not when the indicator method was being used to detect acid production. This was because the fermentation of the carbohydrates in these cases proceeded slowly, as determined by pH determinations after two days' incubation of the cultures, and the indicator was destroyed completely before sufficient acid was produced to cause preceptible changes in the color of the indicator.

Data obtained in the study of the fermentation reactions of five putrefactive anaerobes are shown (Table 1); the pH of the uninoculated medium is reported as a matter of interest. Judgment as to whether or not acid production from the test carbohydrate material had taken place was based upon a significant reduction in pH value between the pH of the inoculated medium less the test material at the end of six days' growth and the pH of the inoculated medium containing the test material at the end of six days' growth.

SEROLOGICAL RELATIONSHIP

The serological relationship of *Cl. sporogenes* (Spray), P. A. 3679, and putrefactive anaerobes S_2 and 9x was determined. Antisera were prepared from three rabbits. One rabbit had received six intravenous injections (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml.) of a 48-hour, whole-broth culture of actively growing putrefactive anaerobe S_2 at six-day intervals. A second rabbit had received similar treatment with P. A. 3679 and a third with *Cl. sporogenes* (Spray). Agglutination of putrefactive anaerobes S_2, 9x, P. A. 3679, and *Cl. sporogenes* by each of the three antisera is recorded (Table 2).

With respect to agglutination reactions, organisms S_2, 9x, and P. A. 3679 would appear to be serologically identical. All are apparently serologically distinct from the strain of *Cl. sporogenes* (Spray). The minor reactions encountered at low-serum dilutions are not considered of differential value.

MORPHOLOGICAL AND CULTURAL CHARACTERISTICS

The following outline describes the cultural and morphological characteristics of S_2, 9x, and P. A. 3679:

Vegetative cells: mediums used, North's gelatin agar, dextrose brain broth; temperature, 37°C.(98.6°F.); age, 48 hours; form, rod with rounded ends; arrangement, majority single, occasionally in short chains; size 0.4 to 0.8 by four to eight microns.

Relation to oxygen: anaerobic.

Endo spores: present; location, subterminal to terminal; oval, distinctly swelling rods.

Motility: positive.

Gram stain: positive.

Nutrient broth: fairly abundant growth, diffuse turbidity, foul odor.

Deep-agar colonies: lenticular to slightly irregular.

Agar-surface colonies: circular, opaque, slightly filamentous.

Gelatin: liquefied completely in 48 hours at 37°C.(98.6°F.).

Milk (iron milk, Spray 1936): blackened within five days at 37°C.(98.6°F.), soft coagulum fifth to sixth day, completely digested by 10th day.

Lead-acetate agar: blackened within 48 hours.

Indol: negative.

Nitrates: not reduced.

Brain medium: blackened and digested with foul odor.

Meat: reddened, then darkened, and digested with foul odor.

Fermentation reaction: growth and acid production from dextrose, levulose, maltose, sorbitol, trehalose, dextrin, starch, salicin, and glycerin. Sucrose fermented by 9x and

TABLE 1

Fermentation Reactions Determined by Change in pH Using an L & N Universal Potentiometer and a Glass Electrode

Sugar, carbohydrate, or glucoside	Uninoculated media	*Cl. sporogenes* (Spray)			*Cl. sporogenes* (J-1)			S_2			9x			P. A. 3679		
		pH of control[1]	pH of test[2]	Significant reduction	pH of control	pH of test	Significant reduction	pH of control	pH of test	Significant reduction	pH of control	pH of test	Significant reduction	pH of control	pH of test	Significant reduction
Aesculin	6.48	5.51	6.64	.....	5.68	6.50	.....	5.71	6.83	.....	5.56	6.71	.....	5.64	6.73	.....
l-Arabinose	6.52	5.55	6.78	.....	5.72	6.78	.....	5.75	6.99	.....	5.60	6.93	.....	5.68	6.88	.....
Adonitol	7.59	6.62	7.10	.....	6.79	6.96	.....	6.82	7.24	.....	6.67	7.03	.....	6.75	7.02	.....
Melibiose	7.52	6.55	6.90	.....	6.72	6.88	.....	6.75	7.15	.....	6.60	6.97	.....	6.68	7.02	.....
Melezitose	7.73	6.76	7.03	.....	6.93	6.94	.....	6.96	7.14	.....	6.81	7.01	.....	6.89	7.07	.....
d-Mannose	7.33	6.36	6.65	.....	6.53	6.67	.....	6.56	6.97	.....	6.41	6.78	.....	6.49	6.81	.....
d-Galactose	7.20	6.23	6.79	.....	6.40	6.80	.....	6.43	6.75	.....	6.28	6.78	.....	6.36	6.60	.....
d-Mannitol	7.73	6.76	6.77	.....	6.93	7.07	.....	6.96	7.05	.....	6.81	7.04	.....	6.89	6.90	.....
Dulcitol	7.72	6.75	7.12	.....	6.92	7.06	.....	6.95	7.11	.....	6.80	7.06	.....	6.88	7.02	.....
Cellobiose	7.50	6.53	6.43	.....	6.70	6.68	.....	6.73	7.20	.....	6.58	6.89	.....	6.66	6.79	.....
d-Fructose	7.37	6.40	6.00	0.40	6.57	4.74	1.83	6.60	5.43	1.17	6.45	5.45	1.00	6.53	5.66	0.87
Inulin	7.72	6.75	7.05	.....	6.92	5.98	0.94	6.95	7.35	.....	6.80	7.15	.....	6.88	7.17	.....
Maltose	7.53	6.56	5.78	0.78	6.73	4.66	2.07	6.76	5.97	0.79	6.61	5.89	0.72	6.69	5.97	0.72
l-Rhamnose	7.48	6.51	6.88	.....	6.68	6.75	.....	6.71	7.01	.....	6.56	6.94	.....	6.64	6.92	.....
Raffinose	7.75	6.78	6.88	.....	6.95	7.04	.....	6.98	7.17	.....	6.83	7.03	.....	6.91	7.07	.....
Starch	7.77	6.80	6.97	.....	6.97	7.12	.....	7.00	6.55	0.45	6.85	6.48	0.37	6.93	6.50	0.43
Sucrose	7.74	6.77	6.75	.....	6.94	7.04	.....	6.97	6.84	.....	6.82	5.32	1.50	6.90	5.81	1.09
d-Sorbitol	7.75	6.78	6.18	0.60	6.95	6.40	0.55	6.98	6.44	0.54	6.83	6.08	0.75	6.91	6.45	0.46
Trehalose	7.78	6.81	5.12	1.69	6.98	5.91	1.07	7.01	6.10	0.91	6.86	6.08	0.78	6.94	6.01	0.93
Salicin	7.80	6.83	6.50	0.33	7.00	6.78	0.22	7.03	6.52	0.51	6.88	6.33	0.55	6.96	6.37	0.59
i-Inositol	7.82	6.85	7.06	.....	7.02	6.90	.....	7.05	7.08	.....	6.90	7.13	.....	6.98	6.96	.....
Glycerol	7.85	6.88	7.00	.....	7.05	6.92	.....	7.07	6.52	0.55	6.93	6.53	0.40	7.01	6.44	0.57
Dextrin	7.80	6.83	6.94	.....	7.00	6.97	.....	7.03	6.41	0.62	6.88	6.44	0.44	6.96	6.35	0.61
l-Xylose	6.84	5.86	6.77	.....	6.03	6.72	.....	6.06	6.74	.....	5.91	6.84	.....	5.99	6.69	.....
d-Glucose	7.36	6.93	5.14	1.79	6.56	5.76	0.80	6.59	5.73	0.86	6.44	5.65	0.79	6.52	5.63	0.89
d-Lactose	7.63	6.66	6.99	.....	6.83	7.07	.....	6.86	6.99	.....	6.71	7.06	.....	6.79	6.92	.....
None	7.83	6.86	6.86	.....	7.03	7.03	.....	7.06	7.06	.....	6.91	6.91	.....	6.99	6.99	.....

[1] Control—inoculated medium less carbohydrate test material at the end of six days' growth. [2] Test—inoculated medium plus carbohydrate test material at the end of six days' growth.

TABLE 2

Agglutination Reactions

Serum dilution	Anti-S_3 serum				Anti-P. A. 3679 serum				Anti-*Cl. sporogenes* (Spray) serum			
	S_3	9x	P. A. 3679	*Cl. sporogenes* (Spray)	S_2	9x	P. A. 3679	*Cl. sporogenes* (Spray)	S_2	9x	P. A. 3679	*Cl. sporogenes* (Spray)
1-20	4+	4+	4+	1+	4+	4+	4+	1+	1+	1+	0	4+
1-40	4+	4+	4+	0	4+	4+	4+	0	1+	1+	0	4+
1-80	4+	4+	4+	0	4+	4+	4+	0	1+	0	0	4+
1-160	4+	4+	4+	0	4+	4+	4+	0	0	0	0	4+
1-320	4+	4+	4+	0	4+	4+	4+	0	0	0	0	4+
1-640	3+	3+	3+	0	3+	3+	4+	0	0	0	0	4+
1-1280	2+	2+	1+	0	2+	2+	2+	0	0	0	0	3+
1-2560	0	1+	0	0	0	1+	0	0	0	0	0	0
1-5120	0	0	0	0	0	0	0	0	0	0	0	0
1-10240	0	0	0	0	0	0	0	0	0	0	0	0

P. A. 3679 while S_2 does not ferment. Not fermented: lactose, xylose, inositol, raffinose, rhamnose, inulin, cellobiose, dulcitol, mannitol, adonitol, arabinose, aesculin, melibiose, melezitose, mannose, and galactose.

Temperature: growth most rapid at 37°C.(98.6°F.); spores, high heat resistance.

Toxicity: whole culture (glucose-beef extract broth with proteose-peptone) neither toxic nor pathogenic to guinea pigs and rabbits by feeding or injection.

Source: spoiled comminuted canned meat.

SUMMARY

Putrefactive anaerobes S_2 and 9x isolated as the causative agents in spoilage of canned meats and S_2 used as a test organism in this series of investigations are shown to be culturally and serologically identical with P. A. 3679, a test organism that has been frequently used by the N. C. A. laboratories for inoculated-pack studies.

Further, S_2, 9x, and P. A. 3679 are shown to be culturally and serologically distinct from a strain of *Cl. sporogenes* (Spray).

The morphological and cultural characteristics of S_2, 9x, and P. A. 3679 are described.

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BACTERIOLOGICAL STUDIES RELATING TO THERMAL PROCESSING OF CANNED MEATS

VI. THERMAL DEATH-TIME CURVE FOR SPORES OF TEST PUTREFACTIVE ANAEROBE IN MEAT

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The test organism used in this study was putrefactive anaerobe S_2 (P. A. 3679) that has been used continuously during this series of investigations. Gross, Vinton, and Stumbo (1946) reported that S_2 was identical with P. A. 3679 used by the Research Laboratories of the National Canners' Association as a test organism for inoculated-pack studies. Therefore, the slope of the thermal death-time curve (z value) is of considerable importance because it must be known for a given organism in order to determine the lethality of a given thermal process with regard to that organism. When using either the methods of Bigelow, Bohart, Richardson, and Ball (1920) or Ball (1928) for evaluating lethality the thermal death-time curve must be known or assumed so it becomes highly important to determine this relationship especially for an organism being used as a test organism for studying the effect of various factors upon thermal processing. It has been common practice to assume a value of 10°C.(18°F.) for z in those cases where z may be unknown. Unfortunately, z is usually unknown because in many cases the organism which may be the causative agent in the spoilage and therefore the organism for which the thermal process is designed is not known. In this particular study this is not true because we have purposely used a test organism of heat resistance well above that of any bacterium naturally occurring in or contaminating the product. Canned-meat products containing other than meat and curing agents would be an exception to this statement and the picture would then be complicated by the possible organisms contained in the added substances other than meat.

Other investigators have studied this problem but to only a limited extent in a meat medium. Williams (1940) reported the resistance of P. A. 3679 in the range of 15 to 16 minutes at 115°C.(239°F.). Cameron (1940) reported values for z in various products including meat ranging from 8.9 to 13.3°C.(16 to 24°F.). Sognefest and Benjamin (1944) reported for 16,000 P. A. 3679 spores suspended in pea purée at pH 5.5 in thermal death-time cans the values of 5.5 and 16 for F and z, respectively. Townsend, Esty, and Baselt (1938) reported values of z from 16.6 in phosphate buffer at pH 7.0 to 20.0 in whole milk and with corresponding F values of 4.0 and 2.6.

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Inasmuch as there still appears to be some question as to the value of z for P. A. 3679 at least in a product such as meat, and inasmuch as the z value is believed to be affected by various factors, it was thought worth while to attempt to determine the z value for S_2 (P. A. 3679) spores in meat and in meat with various commonly used curing agents. In this series of investigations to date the z value has been assumed to be 18°F. as is customary practice when the exact value is unknown. An investigation was started to check this value and the work was completed at about the time of publication of the work of Sognefest and Benjamin (1944). Since that time this work has been repeated.

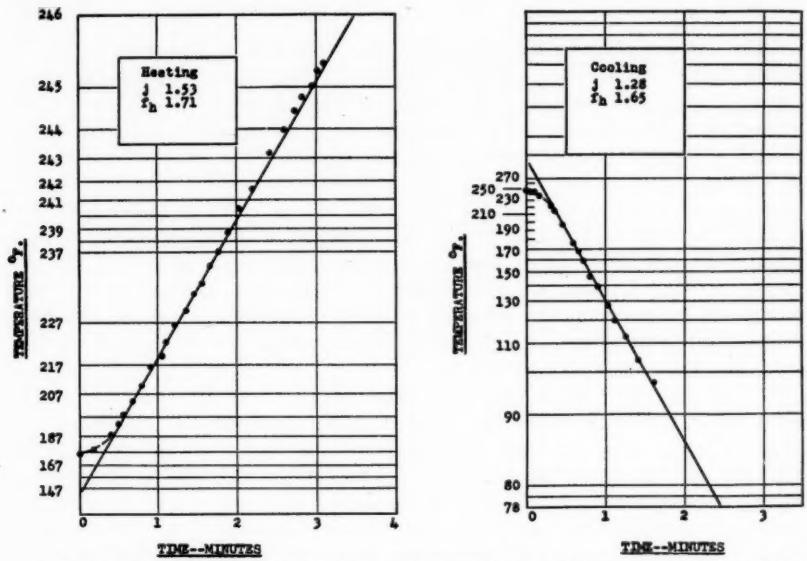

FIG. 1. Rate of heat penetration and cooling meat in 10 x 75 mm. chemical test tubes. RT, 237°F.; CW, 70°F.; IT, 175°F.

EXPERIMENTAL PROCEDURE

The technique used was that described by Stumbo, Gross, and Vinton (1945). The spore suspension used was the identical one described. It should be noted that the technique was designed to compare as closely as possible to commercial practice. Using this technique the determination of thermal death-time is made in the same substrate as in the commercial canning practice which the experiment is designed to study. It is believed extremely important by the authors that such a condition be realized so that results on the laboratory scale can be translated into practice with a greater degree of assurance that they are directly applicable.

Determinations of the rate of heat penetration in meat using 10 x 75 mm. chemical test tubes as thermal death-time tubes has been reported by Stumbo, Gross, and Vinton (1945). A typical heating and cooling curve is shown (Fig. 1); previously a slope of z equals 18°F. was assumed,

but now that the slope is known to be in the range of 8.3 to 8.9°C. (15 to 16°F.) an average value of 15.6°F. was used to determine the correction for heating lag using the general method in the manner suggested by Schultz and Olson (1940). The value obtained agrees within the experimental error with the corrections obtained using z equals 18°F. It should be noted that the heating curve (Fig. 1) for meat is a straight-line function at least to 118.9°C.(246°F.).

The thermal death-time curves for 7,500 to 10,000 spores of S_2 (P. A. 3679) in meat alone and with various curing agents used in maximum amounts permitted for packing houses operating under federal inspection are shown (Fig. 2). These values were obtained using the end-point technique and the curves are plotted showing last positive and first negative values. The values shown are the average values obtained in several experiments.

TABLE 1

Comparison of F Values at 121.1°C.(250°F.) Determined and Obtained by Extending the Straight Lines in Fig. 2.

Substrate inoculated with 7,500 to 10,000 spores of S_2	Extended straight line	Actually determined	z value of straight line
Meat–sugar 1%	6.8	12-13	15.1
Meat–sugar 1%–nitrite 0.016%	4.9	8-9	15.0
Meat–sugar 1%–nitrate 0.17%	8.2	11-12	15.6
Meat–sugar 1%–salt 3½%	9.2	16-....[1]	16.2
Meat–sugar 1%–salt 3½%–nitrite 0.016%–nitrate 0.17%	6.7	7.5-8.5	15.8

[1] End point of destruction not reached.

Table 1 lists the F values at 121.1°C.(250°F.) as determined by prolonging the straight lines in Fig. 2 and by experimental determination. It is readily apparent that the values do not agree.

In order to determine whether or not the number of spores present had any effect upon the slope of the thermal death-time curve, experiments were made using 10 spores per gram. The results obtained were a z value of 14.5 and an F value of 2.6 at 250°F., whereas the experimental data gave an F value of 5.7 at 250°F. The effect of curing agents was also determined at a 10-spore inoculation level and it was concluded that salt, sodium nitrite, sodium nitrate, and sugar in concentrations the same as shown (Table 1) do not affect the slope of the thermal death-time curve within the experimental error of about 10 per cent.

DISCUSSION

The heat-penetration curve (Fig. 1) is a straight line through 246°F. and therefore the conclusion is justified that the rate of heat penetration in the meat is normal and proceeds by conduction at least through 246°F. Any effect noted on the thermal death-time at a temperature of 245°F. is not due to abnormal heating.

In range-finding experiments it was noted that F values at 250°F. were not in line with those at 104.4, 110, and 115.6°C.(220, 230, and 240°F.) so an additional temperature of 118.3°C.(245°F.) was used. Values for ther-

mal death-time at 220, 230, 240, 245, and 250°F. give sufficient points so that in Fig. 2 fairly reliable curves can be drawn. It is obvious that there is a considerable deviation from a straight-line function at temperatures above 240°F. Tanner (1944), Rahn (1930), and others have pointed out that thermal death-time curves are not pure logarithmic functions as was originally believed by Bigelow (1921). The recorded literature often shows rather poor agreement between the determined points and a straight line, especially at the higher temperatures. At temperatures of 240 and 245°F. the possible error owing to poor temperature control and the relatively small magnitude of the correction for heating lag are not so great as to cause undue errors in the data. At temperatures of 250°F., and more especially above 250°F., these factors are so great as to seriously impair the precision of the data. In other words, the difficulties of technique at 250°F. and over are very important and the results obtained are not so reliable as at lower temperatures. Owing to the large possible errors inherent in the technique there has been a tendency to discount deviations at the higher temperatures. It is the opinion of the authors that more attention should be given to this point and that mathematical calculations should be based on thermal death-time curves only over that portion of the curve which agrees very closely with a straight line. For example, it is the opinion of the authors that mathematical calculations (Fig. 2) should be based upon data from these curves only over the range 220 to 240°F. It is evident that there is lack of agreement between experimental and calculated values at 250°F. (Table 1). Considerable error can creep into calculations by an assumption of a straight-line, thermal death-time curve when there is deviation to the extent indicated (Fig. 2) at temperatures of 250°F. These comments do not apply if the general methods of calculation are used for data derived from the experimentally determined, thermal death-time curves (not necessarily straight lines) and the experimentally determined curves of heating and cooling. More experimental work on the determination of thermal death-time curves in the product in question should be done. Likewise, greater attention should be given to determining values at sufficient points so that an accurate curve can be drawn. This is especially true at any critical points such as are indicated between 240 and 250°F. (Fig. 2) for this particular organism in a meat substrate.

It should be pointed out that the temperatures involved in this discussion would be those determined at the geometric center of the container and are not processing temperatures. Fortunately, few canned-meat products are processed to reach internal temperatures over 240°F. at the geometric center of the container, and therefore, at least in the case of the type of meat product used in this study, the discussion as to possible errors above 240°F. is only of theoretical interest.

It is a well-known fact that the concentration of spores directly affects the thermal process necessary to destroy them. Esty and Meyer (1922) furnish convincing evidence on this subject in connection with the destruction of spores of *Clostridium botulinum* by heat. Townsend, Esty, and Baselt (1938) commented that the extremely high resistance data obtained by Esty and Meyer (1922) for *Cl. botulinum* might be due in part to the

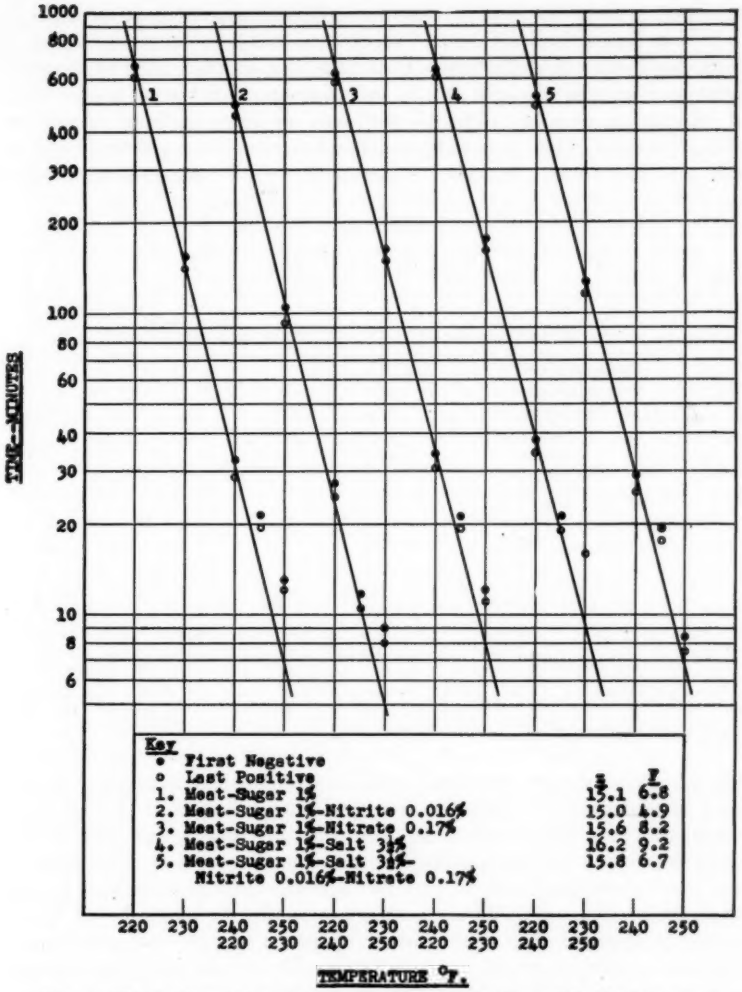

FIG. 2. Effect of curing agents on the slope of the thermal death-time curve.

tremendously large number of organisms used in their resistance determinations. It was decided to investigate the effect of numbers upon the destruction of spores of putrefactive anaerobe S_2 suspended in meat. There was a considerable volume of data already available on this subject. When this data had been summarized and averages obtained by statistical treatment, a curve was drawn comparing the number of spores present with the thermal processes required to destroy them. It was found that the curve might be represented by the equation $y = Ae^{Bx}$ when A, B, and e are constants, y is the number of spores of S_2 per gram of meat, and x is the thermal process value expressed in number of minutes at 250°F.

required to destroy the organism assuming instantaneous heating and cooling. It was found that for spores of S_2 suspended in meat the values of the constants were A is 0.000056, B is 2.02, and e is the base of Naperian logarithms. It was thought to be of theoretical interest to determine what thermal processes might be required to destroy from one to one billion organisms; these calculations were made and the results are shown (Table 2). The column headed "experimental data" shows several values

TABLE 2

Thermal Death-Time for Various Concentrations of Spores of S_2 in Meat

Theoretical calculations [1]		Experimental data
y	x	
1,000,000,000	15.1	....
100,000,000	14.0	....
10,000,000	12.8	....
1,000,000	11.7	....
100,000	10.6	....
50,000	10.2	....
10,000	9.4	9.5
1,000	8.3	8.2
500	7.9	7.8
100	7.1	7.1
50	6.8	....
10	6.0	6.1
5	5.7	4.8
1	4.8	....

[1] Based on equation $y = Ae^{Bx}$ when y is the number of spores per gram; x is the thermal process required for the destruction expressed in minutes at 250°F. assuming instantaneous heating and cooling; A and B are constants having values of 0.000056 and 2.02, respectively, while e is the base of Naperian logarithms.

which are the averages obtained from a statistical treatment of a large volume of experimental data. It is not too surprising that the agreement between the theoretical calculations and the experimental data is good because the values for the constants in the equation were derived from this data. It is interesting to note that the agreement is good over the rather wide range of five to 10,000 organisms per gram. It would be of interest to continue work of this nature with regard to the thermal processes required to destroy spores of *Cl. botulinum*. This work is reported largely as a matter of interest and the authors would like to have it understood that they do not consider this a finished piece of work but rather in the nature of an interesting observation and that it would require a great deal more experimental work and statistical treatment of existing data in order to determine whether this type of treatment can be applied to bacteriological data in general. The data emphasize the fact that the concentration of spores selected for a given experiment directly affects the results obtained insofar as level of heat resistance is concerned.

SUMMARY

The rate of heat penetration in meat using 10 x 75 mm. chemical test tubes as thermal death-time tubes was shown to be a normal straight-line

function through a temperature of 118.9°C.(246°F.) when the logarithm of temperature is plotted against time.

The thermal death-time curve was shown to deviate from a straight-line function above temperatures of 240°F. when the logarithm of time was plotted against temperature. Further, the thermal death-time curve was shown to be a close approximation to a straight line between temperatures of 220 and 240°F.

The z value for the thermal death-time curve of 7,500 to 10,000 spores of S_2 (P. A. 3679) in meat was not affected by the curing agents used in maximum percentages permitted for federally inspected packing houses and the average value of z was 15.6°F. between temperatures of 220 and 240°F. in meat.

The z value was unaffected within the experimental error of about 10 per cent by the concentration of spores present as shown by the z value of 14.2 for 10 spores of S_2 in meat alone or with the maximum percentages of curing agents permitted for use in federally inspected packing houses.

It is suggested that process calculation by the methods of Ball (1928) for a given product be made over a wide range of temperatures only if it can be determined that the thermal death-time curve for a given organism in the given product can be shown to be a straight-line function through the temperature range in question; if the curve is not a straight line over the desired range the general method suggested by Bigelow (1920) may be used, obtaining data from the actual thermal death-time curve drawn through the points determined by experiment. Data are presented to show the discrepancies possible in this particular case when such a possibility was ignored (Table 1).

It is also suggested that wherever possible the thermal death-time curve be determined in the given product for the most resistant spoilage organism which is known or which might be assumed to be present.

The thermal processes required to destroy the spores of S_2 suspended in meat vary as a function of the concentration of spores present. It is suggested that the relationship can be represented by the type equation $y = Ae^{Bx}$. Data calculated from this equation are presented showing the thermal process required for the destruction of from one to one billion spores of S_2 (P. A. 3679) in meat, and experimental data for the destruction of from five to 10,000 spores of S_2 (P. A. 3679) in meat agree well with the calculated values. It is further suggested that this relationship is presented only as a matter of theoretical interest and it should be emphasized that more experimental work is needed to establish the possibility of application of such treatment to bacteriological data in general.

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VITAMIN CONTENT OF DEHYDRATED MEATS[1]

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Large quantities of meat have been dehydrated during the war period. Many of these products have been stored for considerable periods of time or shipped over long distances. It is important, therefore, to know not only the composition of the meat shortly after processing but to follow the retention of the vitamins during storage.

This study has been limited largely to the proximate composition and the thiamin, riboflavin, and niacin content of commercial samples of dehydrated pork and corned-beef hash immediately after production and after definite periods of storage. A few folic acid and biotin determinations have also been included.

EXPERIMENTAL PROCEDURE

The samples were collected in Chicago by workers at the University of Chicago where the proximate analyses, thiamin, and some of the riboflavin determinations were made. Aliquots were taken from each sample and sent to the University of Wisconsin for the other analyses.

Proximate analyses were made according to *Methods of Analysis*, fourth edition, of the Association of Official Agricultural Chemists.

Thiamin—method of Hennessy (1942).

Riboflavin—method of Snell and Strong (1939) at Wisconsin and Peterson, Brady, and Shaw (1943) at Chicago.

The samples for microbiological assay were hydrolyzed with 50 c.c. of 0.1 N HCl at 15 pounds pressure for 20 minutes and cooled, after which two grams of papain dissolved in five c.c. of 2.5 M sodium acetate were added and the mixture incubated at 37°C.(98.6°F.) overnight under toluene. This digest was then neutralized, filtered, and extracted by shaking with ether and the aqueous solution made to volume, according to McIntire, Schweigert, Henderson, and Elvehjem (1943).

Niacin—samples prepared and assayed according to the procedure of Krehl, Strong, and Elvehjem (1943).

Biotin—method of Shull, Hutchings, and Peterson (1942) with the modified medium reported by Shull and Peterson (1943). The samples were hydrolyzed by autoclaving with 4 N sulfuric acid for two hours at 15 pounds pressure.

Folic acid—samples assayed for *S. faecalis* activity against a standard of solubilized liver and the results were then expressed in terms of vita-

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min B_c. The method of Luckey, Briggs, and Elvehjem (1944) was used with the modification suggested by Teply and Elvehjem (1945). Thymol blue was used as the indicator. Samples were prepared by placing five gm. in 40 c.c. of one-per cent sodium acetate buffer pH 4.5-4.7, adding five c.c. of buffer containing 0.1 gm. of takadiastase, and incubating under toluene at 37°C. for 24 hours. The samples were then neutralized and autoclaved at 15 pounds pressure for 15 minutes.

Typical production samples were studied by obtaining one can of dehydrated pork, through the agency of the War Food Administration, at intervals of approximately two weeks, starting in September, 1943, from 14 different manufacturing plants. A total of 14 samples was obtained from each of six plants and seven to eight samples were obtained from each of the other eight plants. These samples were picked at random from the commercial production of each manufacturer by government inspectors. The last samples were collected in May, 1944.

In order to conserve space and because the composition of the individual samples from the same plant was remarkably constant, values are given for only five samples from four different plants. These results are typical of all those obtained. The dry-matter content of these samples was about twice that of fresh pork [McIntire *et al.* (1943); Greenwood, Kraybill, Feaster, and Jackson (1944); Rice and Robinson (1944)] but the dry matter consisted of more protein and less fat than that of fresh pork. The values for protein and fat fall within the same range as those given by Rice and Robinson (1944) for dehydrated pork. If there had been no loss of vitamins during processing the vitamin content should have been two to three times that of fresh pork. This was true for all samples in the case of riboflavin and niacin and indicates little or no loss of these vitamins during processing. Very few of the thiamin values approach a level twice that of fresh meat and many of them were about equal. Thus there was considerable loss of thiamin during dehydration but the loss did not exceed 50 per cent except in the case of Plant C. All the samples from this plant were consistently low in thiamin. Rice and Robinson (1944) reported a retention in the dehydrated meat of 63 per cent of the thiamin originally present in the raw meat. Since fresh pork contains about 8γ of folic acid per 100 gm., or at least 16γ per 100 gm. on the dry-weight basis, the few figures presented indicate a loss of 80 to 90 per cent of the folic acid during processing.

For the storage studies one case, 48 cans, of dehydrated pork was obtained from each of four producers, A, B, C, and D. One case of dehydrated corned-beef hash was also included in the series. Proximate analyses and thiamin, riboflavin, and niacin determinations were made on each product immediately and samples from each case were stored at —17.8, 4.4, 21.1, 37.8, and 48.9°C.(0, 40, 70, 100, and 120°F.), respectively. Vitamin determinations were then made at intervals of one week, 30 days, 10, 20, and 42 weeks on the samples stored at each temperature.

The proximate composition is not given since the values were very similar to those shown (Table 1); the results of the vitamin assay are given (Table 2). It is evident that considerable quantities of thiamin may be lost during storage, especially at the higher temperatures. As would

be expected, the thiamin was stable in all the products when stored at 0°F. There was no significant change in any of the samples stored at 40°F., although the values showed some slight reduction after 42 weeks. In the case of pork definite loss occurred at 70°F. The change was obvious after 30 days and continued to increase with time until the 42-week period, when the loss was very large. At 100°F. storage the loss was apparent at one week, significant at 30 days, and almost complete at 10 weeks. At 120°F. there was a significant loss at one week and almost complete loss at 30 days and after. The changes in the corned beef were much the same although it appears that the thiamin was a little more stable at the 70°F. storage in the beef than in the pork. Values for Plant C were not included in the table because the original value was so low. However, the rate of loss was much the same as that in the other samples.

TABLE 1

Composition of Dehydrated Pork

Plant	Sample No.	Moisture	Fat	Protein	Thiamin	Riboflavin	Niacin	Folic acid
		pct.	*pct.*	*pct.*	*mg. pct.*	*mg. pct.*	*mg. pct.*	*mg. pct.*
A	1	8.2	38.4	52.3	0.82	.69	11.3	2.4
	2	8.6	38.3	50.3	1.10	.60	10.9	.....
	3	8.3	36.3	51.4	0.74	.62	10.8	.....
	4	9.8	41.3	48.8	0.84	.65	9.6	.....
	5	9.5	37.6	50.8	1.10	.64	11.5	.....
B	1	8.1	40.4	48.4	1.10	.65	10.1	1.5
	2	9.7	38.8	49.6	0.69	.62	10.3	.....
	3	8.9	42.5	45.6	0.77	.63	10.3	.....
	4	7.6	43.4	47.2	0.67	.66	9.9	.....
	5	9.2	44.1	45.9	0.68	.71	9.2	.....
C	1	8.0	36.0	54.8	0.28	.76	8.9	1.1
	2	5.0	43.9	47.0	0.38	.76	10.4	.....
	3	6.1	40.2	50.5	0.14	.63	9.5	.....
	4	5.8	42.4	.....	0.27	.76	11.6	.....
	5	3.2	49.9	44.2	0.27	.66	8.7	.....
D	1	3.5	40.9	.....	.....	.65	10.4	3.5
	2	4.5	41.6	48.8	1.40	.66	11.0	.....
	3	6.4	40.0	50.6	0.81	.64	10.1	.....
	4	6.8	43.0	49.6	0.78	.57	11.1	.....
	5	4.0	40.1	53.2	0.65	.68	11.5	.....

The data show that there was no obvious loss of riboflavin and niacin in any of the samples at any of the temperatures. The results for Plants B and C were omitted in order to save space because the results were identical with those presented. The small variations that do appear from sample to sample are undoubtedly due to the difficulty of getting completely homogenous samples.

A few values given (Table 3) indicate no loss of biotin or folic acid during storage even at a temperature of 120°F. for 20 weeks. The stability of biotin is not surprising but it is interesting to note little or no additional loss of folic acid during storage in spite of the large loss during dehydration.

TABLE 2

Effect of Storage at Different Temperatures on Vitamin Content of Dehydrated Meats

(All values given as mg. per 100 gm.)

Plant	Temperature	Original	1 Week	30 Days	10 Weeks	20 Weeks	42 Weeks
				THIAMIN			
	°F.						
A	0	0.85	.....	0.65	.....	0.93	0.87
	40	0.85	.....	1.10	.....	0.78	0.59
	70	0.85	0.84	0.51	0.46	0.34	0.09
	100	0.85	0.64	0.28	0.05	0.00	0.00
	120	0.85	0.13	0.00	0.00	.....	.....
B	0	0.89	.....	1.13	.....	1.02	0.79
	40	0.89	.....	0.66	.....	0.85	0.53
	70	0.89	0.86	0.71	0.46	0.28	0.06
	100	0.89	0.49	0.12	0.16	0.00	0.00
	120	0.89	0.15	0.00	0.00	.....	.....
D	0	1.40	.....	1.58	.....	1.33	1.52
	40	1.40	.....	1.54	.....	1.42	1.33
	70	1.40	1.40	1.13	1.23	1.05	0.68
	100	1.40	1.22	0.84	0.05	0.03	0.00
	120	1.40	0.58	0.10	0.00	.....	.....
Corned-beef hash	0	0.11	.....	0.12	.....	0.09	0.10
	40	0.11	.....	0.09	.....	.....	0.08
	70	0.11	0.07	0.10	0.10	0.12	0.08
	100	0.11	0.06	0.01	0.01	0.01	0.00
	120	0.11	0.01	0.00	0.00	.....	.....
				RIBOFLAVIN			
A	0	0.66	.....	0.65	.....	0.71	0.68
	40	0.66	.....	0.63	.....	0.72	0.66
	70	0.66	0.66	0.68	0.62	0.74	0.72
	100	0.66	0.66	0.64	0.63	0.70	.....
	120	0.66	0.67	0.66	0.64	0.71	.....
D	0	0.72	.....	0.65	.....	0.71	0.65
	40	0.72	.....	0.64	.....	0.66	0.74
	70	0.72	0.63	0.71	0.61	0.72	.....
	100	0.72	0.65	0.69	0.65	0.73	.....
	120	0.72	0.64	0.68	0.67	0.77	.....
Corned-beef hash	0	0.45	.....	0.46	.....	0.50	0.51
	40	0.45	.....	0.41	.....	0.46	0.47
	70	0.45	0.41	0.47	0.41	0.42	.....
	100	0.45	0.45	0.44	0.41	0.45	.....
	120	0.45	0.43	0.44	0.45	0.42	.....
				NIACIN			
A	0	10.7	.....	9.4	.....	10.5	11.3
	40	10.7	.....	11.3	.....	11.2	11.6
	70	10.7	10.1	9.9	10.0	10.1	11.6
	100	10.7	10.0	9.6	10.5	10.7	10.5
	120	10.7	10.5	10.1	9.8	11.2	11.5
D	0	11.2	.....	11.6	.....	11.7	11.8
	40	11.2	.....	12.2	.....	12.0	12.7
	70	11.2	11.6	11.6	11.0	11.2	11.7
	100	11.2	11.5	10.4	10.8	11.2	12.6
	120	11.2	11.6	10.7	10.8	11.5	12.8
Corned-beef hash	0	12.2	.....	11.3	.....	11.4	10.1
	40	12.2	.....	12.1	.....	11.5	10.7
	70	12.2	10.4	10.7	10.9	10.5	11.7
	100	12.2	10.7	10.2	11.3	9.9	12.6
	120	12.2	10.5	9.3	10.4	10.5	9.7

TABLE 3

Effect of Storage on Biotin and Folic Acid Content of Dehydrated Pork

(All values given as micrograms per 100 gm.)

Plant	Original	Stored at 0° F. for 20 weeks	Stored at 120°F. for 20 weeks
	BIOTIN		
A	13.6	13.7	13.0
B	13.2	14.2	12.5
C	13.2	11.8	12.1
	FOLIC ACID		
A	2.2	2.2	1.9
B	2.6	2.1	2.0
C	3.0	3.1	3.6

DISCUSSION

The work reported in this paper, as well as other studies, clearly demonstrates the stability of riboflavin and niacin in meat both during dehydration and during storage of the dehydrated products for periods as long as 42 weeks. Whatever loss does occur is largely dependent upon leaching during the drying process. In the case of thiamin, losses occur during both processing and storage. There is a significant loss of thiamin when the material is stored at room temperatures, and at higher temperatures the loss is serious. The fact that the loss is greater in dehydrated meats than in canned, undried meat products [Rice, Beuk, Kauffman, Schultz, and Robinson (1944)] indicates that the concentration of salts during the dehydration process is undoubtedly responsible for the increased destruction of thiamin. The results for biotin and folic acid are too meager to warrant any generalization but it appears that the stability of biotin is more like that of riboflavin and niacin, and that folic acid is more like that of thiamin.

SUMMARY

Data are presented to show the proximate composition and thiamin, riboflavin, and niacin contents of commercial samples of dehydrated pork and corned-beef hash immediately after manufacture, and the retention of the vitamins during storage of these products at 0, 40, 70, 100, and 120°F. for varying periods of time.

No significant loss of riboflavin and niacin occurred even at the highest temperatures and the longest periods of storage. The thiamin was stable at 40°F., but at 70°F. there was a definite loss after 30 days and a large loss after 42 weeks. At 100 and 120°F. the loss was apparent after one week and almost complete after 10 weeks' storage.

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B-COMPLEX VITAMINS IN MEAT

I. THIAMIN AND RIBOFLAVIN CONTENT OF RAW AND COOKED PORK[1]

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Pork is recognized as an excellent source of thiamin and a fair source of riboflavin. There are several factors, however, that affect the thiamin and riboflavin content of pork as it is consumed by the public. Among these factors are individual differences in the animal and differences in storing, cooking, and processing. Some investigations have been made upon these factors. Christensen, Latzke, and Hopper (1936) reported a 12-per cent loss of thiamin in pork cooked at 90°C.(194°F.), but heating for 70 minutes at 10 lb. pressure destroyed 21 per cent. They found no loss of riboflavin by cooking or autoclaving. Arnold and Elvehjem (1939) showed that pork lost over 80 per cent of thiamin during one hour and 50 minutes of commercial processing at 121°C.(249.8°F.). These authors maintained that the greater losses take place during the first hour of cooking.

Frying produced the least loss of thiamin, according to the results of Mickelsen, Waisman, and Elvehjem (1939), while roasting produced a loss of 50 to 60 per cent of the thiamin. Decreasing the roasting time decreased the thiamin losses. It is possible, according to these authors, that a fried pork chop may supply the daily requirement of thiamin.

A study of the reaction rates for the decomposition of thiamin in pork at various temperature levels was conducted by Rice and Beuk (1945). They found that the rate of loss was constant at any given temperature above 77°C.(170.6°F.) and was proportional to the temperature. The rate of loss decreased below 77°C. during the first 16 to 24 hours and then seemed to remain constant. These investigators pointed out that the holding of foods at steam-table temperatures may not cause as high thiamin losses as is generally supposed. Jackson, Crook, Malone, and Drake (1945) showed that the more the pork is cooked, the greater the thiamin losses. They maintain that the variation between the different cuts in a carcass and between the right and left sides of a carcass is much less than the variation between the different carcasses.

Aughey and Daniels (1940) found that 57 per cent of the thiamin was retained in roast pork and 85 per cent in braised loin. A wide variation in the thiamin content of different carcasses was reported by McIntire, Schweigert, Henderson, and Elvehjem (1943). Their riboflavin values were higher in the cooked samples than in the fresh samples and they attributed this to the loss of water and fat during cooking and a high retention of riboflavin in the cooked meat. The average retention of thiamin

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was 50 per cent after braising and 70 per cent after roasting or broiling, while 75 to 80 per cent of the riboflavin was retained after braising and 80 to 100 per cent by other cooking methods.

Schweigert, McIntire, and Elvehjem (1943) analyzed fresh and cured hams and found the average retention in the meat, when roasted, to be 58 per cent for thiamin and 74 per cent for riboflavin. When the thiamin and riboflavin in the drippings were added to that in the meat the average total retention after roasting was 70 per cent for thiamin and 84 per cent for riboflavin. The thiamin content of the various sections of ham varied considerably but the riboflavin values were more constant. Brady, Peterson, and Shaw (1944) reported a retention of 70 to 80 per cent of the thiamin and 80 per cent of the riboflavin in roasted loin muscles. There were significant differences in the riboflavin content of different muscles from the same pig, according to Peterson, Brady, and Shaw (1943).

That the thiamin content of pork muscle may be influenced by the amount of thiamin in the ration of the animal was shown by Miller, Pence, Dutcher, Zeigler, and McCarthy (1943). Increasing the thiamin in the ration approximately 1.5 times produced a 15- to 20-per cent increase of thiamin in the pork muscle.

Since there are many factors influencing the thiamin and riboflavin content of meat, and there is considerable variation in the losses of these vitamins in the processing of meat, it was considered advisable to obtain further data on this problem. These experiments were designed to study the distribution of thiamin and riboflavin in the pork muscles and their retention under certain cooking and storage conditions.

EXPERIMENTAL PROCEDURE

Thiamin, riboflavin, and moisture analyses were made on both raw and cooked samples of pork to determine the following: (1) sampling problems owing to variation in different parts of the carcass; (2) comparative vitamin content of the right and left side; (3) the effect of cooking by roasting and braising upon the thiamin and riboflavin content; (4) the effect on the thiamin content of holding the roasted meat in the refrigerator overnight, of holding the sliced roasted pork over steam, and of reheating the cold roasted pork.

The pork was obtained from eight experimental animals which had been fed a known ration. One-half portions of two different carcasses were cut into wholesale cuts and the ham was then cut into four pieces and the shoulder into three. The loins, together with those from the other half of the carcass as well as two pairs from other animals, were cut five roasts to a loin. Four pairs of loins were cut four pieces to a loin and a chop one inch thick was cut from each of these but was wrapped with the roast. The meat was double-wrapped in approved freezer-locker paper, frozen, and then stored in a commercial frozen-food locker plant until such time as it was to be analyzed. About 40 hours before a cut was to be used it was transferred to a refrigerator maintaining a temperature of approximately 4°C.(39.2°F.). When removed from the refrigerator the temperature of the meat was about 0°C.(32°F.) although it was often partially frozen.

The vitamin content of the different cuts was determined on the uncooked meat. Samples which were taken from the larger cuts were either a slice which might correspond with a retail cut or an individual muscle. When right and left sides were being compared, uncooked meat was also used and only the longissimus dorsi muscle was tested. For other tests when cooked pork was used the roasts were cooked in an electric oven at 176.7°C.(350°F.) to an internal temperature of 82.2°C.(180°F.) and the chops were braised in a heavy aluminum skillet with a tight-fitting lid to an internal temperature of 182° ± 2° F.

Vitamin analyses were made on the muscle meat from which the bones and gross fat were removed. The thiamin was determined by a modified thiochrome procedure of Conner and Straub (1941). The microbiological method of Snell and Strong (1939), as modified by Strong and Carpenter (1942), was used for the riboflavin determinations. Moisture was determined by the method recommended for the Cooperative Meat Investigations (1940), and all values are reported on the moisture-free basis.

TABLE 1

Thiamin and Riboflavin Content of Comparable Cuts From Two Pork Carcasses

Cut No.	Description	Thiamin [1]		Riboflavin [1]	
		Animal A	Animal B	Animal A	Animal B
		μg./gm.	μg./gm.	μg./gm.	μg./gm.
I	Ham shank	23.0	29.0	8.6	6.1
IIa	Ham slice, near shank[2]	31.9	37.2	8.3	8.0
IIb	Ham slice, intermediate	39.3	56.7	5.9	10.6
IIc	Ham slice, farthest from shank	42.4	46.0	6.1	6.2
IIIa	Adductor muscle, near shank	34.8	40.9	6.7	8.5
IIIb	Adductor muscle, away from shank	34.5	32.5	5.1	6.3
IV	Semitendinosus muscle	33.7	41.6	7.8	8.5
V	Biceps femoris	45.8	53.0	6.7	9.3
VI	Fore shank	17.0	33.4	5.2	9.4
VIIa	Picnic, near shank, ½″ slice	28.0	40.9	6.8	5.8
VIIb	Picnic, intermediate, ½″ slice	32.4	44.1	6.4	9.4
VIIc	Picnic, farthest from shank, ½″ slice	18.5	23.1	6.6	11.0
VIIIa	Boston butt, bottom ½″ slice	20.9	27.5	6.3	6.0
VIIIb	Boston butt, center ½″ slice	24.8	27.2	6.3	6.0
VIIIc	Boston butt, top ½″ slice	21.3	29.2	5.4	5.6

[1] Animal A received 0.30 per cent calcium in the ration. Animal B 0.35 per cent. [2] Ham slice included rectus femoris, vastus internus, vastus externus, and vastus intermedius muscles.

RESULTS AND DISCUSSION

The results of analyses of the different cuts showed those from one carcass to vary in thiamin content from 17.0 to 45.8 μg. per gm. and from the other, 23.1 to 56.7 μg. per gm. (Table 1). Although the cuts highest in thiamin from one carcass were not always the ones which were highest in the other, there was, on the whole, good agreement as is shown by a correlation coefficient of 0.77, a figure far greater than the one-per cent level. The riboflavin content of the two carcasses varied from 5.1 to 8.6 μg. per gm. and 6.0 to 11.0 μg. per gm., respectively. These differences in the two carcasses were of interest since the only known variable in the production of the two animals was in the calcium content of the ration.

This led to further comparison of the longissimus dorsi muscles of seven animals used in the study. The results indicate a range from 17.6 to 33.3 μg. per gm. of thiamin and from 3.9 to 6.3 μg. per gm. of riboflavin (Table 2). It would seem that other factors than diet play a part in determining the thiamin content of the pork muscle, although there was a distinctly higher trend of thiamin in the muscle from the hogs on the higher dietary calcium level.

In the case of the riboflavin there is some indication of a decrease with prolonged frozen storage (Table 2). The cuts having an average riboflavin content of 6.3 and 6.1 μg. per gm. were analyzed within the first year of storage and the remaining cuts within the second year.

TABLE 2

Average Thiamin and Riboflavin Content of Pork Loin

Calcium in ration	Number determinations from animal	Thiamin	Riboflavin
pct.		*μg./gm.*	*μg./gm.*
.30	24	26.4	6.3
.35	20	32.4	6.1
.35	8	24.2	4.1
.35	8	33.3	4.2
.30	4	17.6	4.0
.30	1	24.6	3.9
.30	8	19.5	4.7

TABLE 3

Thiamin and Riboflavin Content of Adjacent Cuts of Paired Longissimus Dorsi Muscles

Loin cut No.	Thiamin				Riboflavin			
	Animal A		Animal B		Animal A		Animal B	
	Right	Left	Right	Left	Right	Left	Right	Left
	μg./gm.	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*
1	26.2	28.3	33.0	31.1	6.9	4.4	5.3	6.1
2	24.2	29.3	33.9	31.2	9.0	8.2	5.3	6.3
3	25.4	24.4	.....	.....	4.4	8.2	....	....
4	24.5	24.5	39.1	32.8	5.8	4.0	5.9	5.2
5	29.1	27.6	33.5	29.0	6.3	5.2	5.3	4.9
6	23.6	26.9	.....	.....	6.3	5.2	....	....
7	24.9	25.6	33.8	34.5	6.3	4.0	6.7	7.3
8	24.7	28.4	34.3	30.8	5.2	4.3	5.9	6.8
9	25.7	25.9	31.9	32.7	5.3	5.6	6.5	7.6
10	26.3	28.1	29.4	29.7	5.8	5.4	5.4	6.5
11	23.4	22.4	26.0	23.8	5.0	4.6	5.3	4.0

The variation of the thiamin and riboflavin content of the longissimus dorsi muscle was relatively small when adjacent cuts were considered (Table 3). No significant difference was found in the left and right sides, although in Animal A, nine out of 11 right-side cuts contained more riboflavin than the corresponding left cuts. In Animal B, six out of nine left cuts contained more riboflavin than the right cuts.

The loins cut into roasts and chops were used to study the comparative losses in roasting and braising. The results (Table 4) indicate the cooked roasts had a slightly higher thiamin content than the chops, although the actual percentage retained was lower. This seeming inconsistency is due to the fact that the uncooked roasts showed a higher thiamin content than the uncooked chops. In a comparison of chops and roasts from different carcasses it was found that the chops were lower in thiamin content in every instance. This difference may have been due to the size of the cut, which in the case of the chop may have resulted in greater loss of thiamin during the prolonged period of frozen storage. The roasts also showed a higher riboflavin content but the percentage retention was 83.1 as compared with 74.2 for the chops, a difference sufficient to account for the greater riboflavin content of the roasts.

TABLE 4

Data on 12 Cooked Pork Roasts and 12 Braised Pork Chops

Factors	Roasts		Chops	
	Range	Average	Range	Average
Weight	780 -1717 gm.	1144 gm.	204 -332 gm.	266 gm.
Cooking loss	20.1- 27.5%	24.0%	14.4-23.3%	18.6%
Moisture content	46.1- 59.7%	53.3%	46.8-58.0%	56.3%
Thiamin content	7.3- 23.2 μg./gm.	14.7 μg./gm.	7.1-19.7 μg./gm.	14.2 μg./gm.
Thiamin retained	41.7- 75.9%	55.3%	50.8-70.0%	59.1%
Riboflavin content	2.7- 4.6 μg./gm	3.5 μg./gm.	2.5- 3.7 μg./gm	3.2 μg./gm.
Riboflavin retained	65.9-104.6%	83.1%	64.4-96.6%	74.2%
Thiamin in drippings	0.0- 4.4%	1.4%	11.9-38.4%	21.2%
Riboflavin in drippings	1.5- 9.0%[1]	5.2%[1]	12.8-34.1%[1]	20.1 %[1]

[1] Eleven determinations. One sample which was unaccountably high was not included.

The effect of holding cooked pork over steam for later service was studied by removing the longissimus dorsi muscle from six cooked pork roasts. Each muscle was cut into two pieces crosswise. One was ground at once and prepared for analysis. The other piece was sliced, water was added to keep it moist, and it was held in an aluminum pan over rapidly boiling water for 30 minutes. The thiamin content of the meat ground at once ranged from 13.0 to 25.5 μg. per gm. (Table 5), while that held over steam varied from 11.2 to 21.5 μg. per gm. and retained an average of 91 per cent of that ground at once.

To determine the effect on the thiamin content of holding cooked pork in the refrigerator, one each of 10 paired pork roasts was analyzed immediately after cooking, the other after storing overnight at approximately 4.4°C.(40°F.). The thiamin content of the meat analyzed immediately after cooking varied from 7.9 to 25.5 μg. per gm.; that held overnight, from 6.8 to 25.8 μg. per gm. (Table 5). The thiamin retained in the pork held overnight averaged 92.8 per cent of that analyzed immediately after cooking.

The longissimus dorsi muscle was removed from six cooked pork loin roasts which had been held in the refrigerator overnight. The muscle was cut crosswise into two parts. One half was ground at once, the other half was sliced, placed in an aluminum pan, water was added, and the meat

reheated in a 350°F. electric oven for 36 minutes. The thiamin content of that held overnight and not reheated ranged from 12.8 to 25.8 μg. per gm. and of that reheated, from 13.5 to 22.6 μg. per gm. (Table 5). The thiamin retained in the pork which had been reheated averaged 93.1 per cent of that which was not reheated.

TABLE 5

Effect of Holding Overnight in Refrigerator and of Reheating Upon Thiamin Content of Roast Pork

Ground at once	Held over steam	Held overnight in refrigerator	Held overnight in refrigerator; reheated	Retention			
				Held over steam	Held overnight	Reheated	Held overnight; reheated
μg./gm.	μg./gm.	μg./gm.	μg./gm.	pct.	pct.	pct.	pct.
9.2	.....	8.4	.....	.....	91.3	.....	.....
14.5	.....	14.1	.....	.....	97.2	.....	.....
16.7	16.5	18.8	15.8	98.8	112.6	84.0	94.6
13.0	13.8	.....	.....	106.2	.....	.....	.....
25.5	21.1	25.8	22.6	82.7	101.2	87.6	88.6
25.4	21.5	23.1	21.6	84.6	90.9	93.5	85.0
16.3	11.2	12.8	13.5	68.7	78.5	105.5	82.8
19.2	20.1	18.7	19.3	104.7	97.4	103.2	100.5
15.9	.....	13.4	.....	.....	84.3	.....	.....
7.9	.....	6.8	.....	.....	86.1	.....	.....
11.7	.....	10.3	.....	.....	88.0	.....	.....
.....	.....	21.0	17.8	.....	.....	84.8	.....
Average	.....	.....	.....	91.0	92.8	93.1	90.3

Five cooked pork loins with a thiamin content from 16.3 to 25.5 μg. per gm. when held overnight in the refrigerator, sliced, and reheated had a thiamin content ranging from 13.5 to 22.6 μg. per gm. When these were compared it was found that the thiamin retained in the reheated loins averaged 90.3 per cent of those ground at once (Table 5).

SUMMARY

Different cuts of pork from the same carcass, with gross fat removed, varied widely in thiamin content and to a lesser extent in riboflavin content.

Loin cuts from experimental animals eating the same ration varied considerably in thiamin content.

Pork roasts and braised pork chops showed practically the same thiamin loss in cooking; however, only about 20 per cent of the thiamin in the chops was destroyed as compared with 43 per cent in the roasts. The roasts retained a higher percentage of riboflavin but had a higher total loss than did the chops.

Pork roasts sliced and held over steam for 30 minutes retained 91 per cent of the thiamin in the cooked meat.

Pork roasts held overnight retained 92.8 per cent of the thiamin present in the freshly cooked roasts.

Pork roasts held overnight, sliced, and reheated retained 93.1 per cent of the thiamin in the roasts held overnight and 90.3 per cent of that in the meat immediately after cooking.

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LEAVES AND STEMS OF TURNIP GREENS AS A SOURCE OF SOME NUTRIENTS[1]

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Custom in preparing turnip greens for cooking varies exceedingly. In some kitchens the entire stems are retained, in others they are completely discarded no matter what the size or age of the greens. In still other kitchens stems of young plants are cooked, while not only the stems (petioles) of larger and older plants are thrown away but midribs also are stripped from the leaf blades. Preferences for greens with stems, or for the leaves only, have been developed largely through the eating of greens prepared in one way or the other. The emphasis now placed upon the conservation of nutritive values and the prominence of turnip greens in the southern diet indicate that it is worth while to determine the comparative potential food values of leaves and stems of this leafy vegetable.

EXPERIMENTAL PROCEDURE

Only one variety of turnip greens, Seven-Tops, was used for this study. With seed from the same source, greens were grown at College Station and Winter Haven, Texas, in 1938[2]; at College Station and Lubbock in 1941. The greens at Lubbock were grown primarily for this purpose; at College Station and Winter Haven they were taken from the guard rows of experimental plantings, one fertilized (300 lb. 6-12-6 per acre), the other not fertilized. The Lubbock samples were grown with supplemental irrigation on a Dalhart fine sandy loam which is generally considered fertile, with a relatively high availability of elements for plant growth since it has been developed under limited rainfall and only recently put under cultivation. Soil type at College Station was Lufkin fine sandy loam; at Winter Haven, Orelia clay loam. The greens were grown under irrigation at Winter

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Haven; those at College Station with a recorded rainfall of 3.54 inches during the growing period. In 1938, the samples, all marketable size, were harvested on the same day at Winter Haven; at College Station, within the same week. In 1941, the samples represented successive stages of growth. Procedure in harvesting the greens and preparing them for analysis was the same as that described in a previous report by Whitacre, Fraps, Yarnell, and Oberg (1944), except with respect to separation of stem and leaf blades. Midribs attached to petioles are called stems in this study. Stems were stripped from the leaf blades, using the hands, immediately after weighing the leaves (stems attached) regarded as suitable for cooking. Weight after washing of stems and leaves separately was used as fresh weight, the greens being then of more nearly uniform turgidity than at any other point of handling. For comparison, samples with stem and leaf blade unseparated were prepared from some of the harvestings in 1941.

Two or more determinations of each constituent were made on each sample. Determination of the content of moisture in fresh material and of calcium, phosphorous, and iron in the dried material was made on all samples. In addition, the samples from Winter Haven were analyzed for total ash, nitrogen, fat, crude fiber, and nitrogen-free extract. Iron determinations were made by a procedure based on the o-phenanthroline method of Saywell and Cunningham (1937). However, nitric acid as well as sulfuric and perchloric acids were used in the wet ashing of the samples, according to the method of Jackson (1938), and a Klett-Summerson (1939) photoelectric colorimeter was used for measuring color concentrations. For all other constituents methods of the Association of Official Agricultural Chemists (1940) were used.

RESULTS AND DISCUSSION

The division of the greens between leaf and stem portions (Table 1) shows that for individual samples the leaf portions range from 48.50 to 75.55 per cent, with corresponding stems making up 51.50 and 24.45 per cent, respectively, of the total weight of those samples. The average values for the 18 pairs of samples are 63.33 per cent leaf, 36.67 per cent stem. These values are in line with data reported by Sheets, Leonard, and Gieger (1941) for Seven-Tops turnip greens grown in one place in Virginia and four places in Mississippi (10 samples in each of the five lots). The averages for these five lots show a range of 39.17 to 58.57 per cent leaves with minimum and maximum values among the 50 individual samples of 33.57 to 64.68 per cent.

The data on percentage content of calcium, phosphorus, and iron expressed both on the dry basis and the fresh and moisture in the fresh greens are shown (Table 2) for the total of 41 samples analyzed—18 of leaves, 18 of corresponding stems, and five samples of leaf plus stem.

Among the 18 pairs of leaf and stem samples, on the dry basis, there were 12 samples of stems which exceeded in calcium content their mate sample of leaves, only two of stems which exceeded leaves in phosphorus while all 18 leaf samples exceeded corresponding stems in iron content. On the fresh basis, relationship of leaf and stem samples respecting calcium

content was almost reversed from that on the dry basis, only three of stem exceeding leaf samples. Plant parts, fresh basis, differed from dry basis less for phosphorus and iron than was true for calcium. Phosphorus content, fresh basis, was greater in leaves of 16 pairs, in stems of one pair, the same in leaves and stems of one pair. In iron, the 18 leaf samples exceeded stem, but in one pair difference was only one in the fourth decimal place. In water content 16 stem samples exceeded corresponding leaves.

TABLE 1

Leaf and Stem Portions of Turnip Greens[1]

Sample	Harvest date	Treatment[2]	Weight (grams)			Percentage of total weight	
			Total	Leaves	Stems	Leaves	Stems
College Station, 1938	Nov. 10	f	672.4	432.3	240.1	64.29	35.71
	Nov. 10	nf	1344.3	846.8	497.5	62.99	37.01
	Nov. 11	nf	1338.4	805.5	532.9	60.18	39.82
	Nov. 11	nf	988.5	650.3	338.2	65.79	34.21
	Nov. 4	nf	1584.4	768.5	815.9	48.50	51.50
	Nov. 10	nf	745.3	522.8	222.5	70.15	29.85
						Av. 61.52	38.48
College Station, 1941	Nov. 4	f	1190.6	793.8	396.8	66.67	33.33
	Nov. 17	f	2380.4	1473.2	907.2	61.89	38.11
	Dec. 1	f	1111.5	636.4	475.1	57.26	42.74
	Jan. 11	f	2785.0	1661.0	1124.0	59.64	40.36
						Av. 61.36	38.64
	Dec. 1	nf	1372.3	892.9	479.4	65.07	34.93
Lubbock, 1941	May 16	nf	795.2	553.1	242.1	69.55	30.45
	May 26	nf	1585.4	1103.9	481.5	69.63	30.37
	May 29	nf	1197.7	588.3	609.4	49.12	50.88
						Av. 62.77	37.23
Winter Haven, 1938	Dec. 1	f	1107.2	692.3	414.9	62.53	37.47
	Dec. 1	f	885.2	556.3	328.9	62.84	37.16
						Av. 62.68	37.32
	Dec. 1	nf	889.4	671.9	217.5	75.55	24.45
	Dec. 1	nf	789.7	539.4	250.3	68.30	31.70
						Av. 71.92	28.08
Average all samples	.....	....	.....	.....	.....	63.33	36.67

[1] Greens were washed, surface was dry, not wilted. [2] f means fertilized; nf, not fertilized.

The maximum content of calcium in the fresh leaves (0.40 per cent) is approximately twice that of the minimum content (0.19 per cent), while the highest value is three times that of the lowest in phosphorus (0.088 and 0.024 per cent) and also in iron (0.0041 and 0.0013 per cent). In fresh stems the highest content of both calcium and phosphorus approximated three times the lowest content (calcium, 0.43 and 0.14 per cent; phosphorus, 0.64 and 0.22 per cent), while the highest iron was eight times the lowest (0.0040 and 0.0005 per cent). Individual samples of leaves plus stems in their content of calcium, phosphorus, and iron (fresh basis) occupied a place between corresponding separated leaves and stems among samples from College Station. But this was true for only three of

nine similar comparisons for samples from Lubbock, the remaining six leaf plus stem samples having the highest content of the given mineral. While there are some irregularities, the trend (fresh basis) is for slight increase of calcium and phosphorus content as the greens mature. Younger plants from College Station contained more iron, those from Lubbock less, than older plants. In fresh greens there may be marked differences in nutritive value dependent upon variation in composition.

Means with standard error, standard deviation, and coefficient of variability were calculated (Table 3) for those groups consisting of three or more samples from greens grown the same year in a given place under the same fertilizer treatment. Coefficients of variability for moisture (range 0.61 to 2.51) are much smaller than for any of the three minerals on the fresh basis, those for calcium ranging from 9.78 to 30.36; for phosphorus, from 9.97 to 28.71; for iron, from 9.26 to 36.60. With a few exceptions the coefficients of variability were from one and one-half to two times as great for fresh samples as for corresponding ones on the dry basis. As a rule, the stems were more variable than the mate sample of leaves although there are sufficient exceptions to be noteworthy; and a number of differences are small, particularly for content of water and iron. Most of the exceptions to the trend of differences between leaves and stems are among the dry-basis samples from Lubbock in 1941. These samples were prepared in the same laboratory as were those from College Station in 1941. The latter conform to the trend. The average proportion of leaves for the three Lubbock samples is 62.76 per cent, for the four College Station samples, 61.37 per cent. There is no statistically significant difference in the water content either of leaves or of stems between samples from Lubbock and College Station. On the fresh basis, however, the leaf and stem relationships among the Lubbock 1941 samples are concordant with other groups of samples, stems varying more than leaves in all constituents except phosphorus for which the coefficient is 21.4 for leaves, 20.0 for stems.

The results of variance analysis applied to all suitable data on calcium, phosphorus, iron, and water are given (Table 4). Out of 35 comparisons of leaves and stems, 20 statistically significant differences were found, all but one of these being at the one-per cent level. Most of these significant differences were with the phosphorus and iron; there were none with calcium on the dry basis and only two out of five analyses on the fresh basis. Stems contained significantly more water than leaves in three of five comparisons. Of the 20 significant differences, 17 were found in the crops of 1938 with similar distribution for College Station and Winter Haven. There were no significant differences between stems and leaves of the greens grown in Lubbock in 1941.

The variation in composition of the leaves and stems of the greens from the different localities is doubtless due in part to differences in soil and climatic conditions, but the limited data do not permit specific explanation of the relationship between environment and composition. Divergence of the Lubbock 1941 samples from the other groups in stem-leaf relationships is not unique, however. Sheets, Leonard, and Gieger (1941) reported that among 40 samples of Seven-Tops turnip greens (10 samples from each of

TABLE 2

Content of Ca, P, and Fe in Individual Samples of Turnip Greens and Averages for Groups of Three or More Samples

Sample	Treatment [2]	Harvest date	Dry basis (per cent)								
			Ca			P			Fe		
			Leaf	Stem	Leaf + stem	Leaf	Stem	Leaf + stem	Leaf	Stem	Leaf + stem
C.S.[1]'38	f	Nov. 10	2.95	3.18	.....	.785	.578	.....	.0247	.0119	.....
C.S.'38	nf	Nov. 10	3.12	3.58	.....	.712	.413	.....	.0209	.0096	.....
		Nov. 11	3.32	3.20	.....	.670	.473	.....	.0275	.0134	.....
		Nov. 11	3.23	3.30	.....	.622	.397	.....	.0194	.0123	.....
		Nov. 4	3.07	3.86	.....	.678	.406	.....	.0242	.0089	.....
		Nov. 10	3.39	3.38	.....	.727	.559	.....	.0180	.0092	.....
		Average	3.23	3.46	.....	.682	.450	.....	.0220	.0107	.....
C.S.'41	f	Nov. 4	2.65	2.79	.....	.575	.614	.....	.0282	.0173	.....
		Nov. 17	2.57	3.14	.....	.672	.533	.....	.0199	.0097	.....
		Dec. 1	2.24	2.46	2.19	.740	.479	.722	.0163	.0084	.0139
		Jan. 11	2.50	2.34	2.38	.554	.363	.437	.0153	.0101	.0144
		Average	2.49	2.68	.....	.636	.497	.....	.0199	.0114	.....
C.S.'41	nf	Dec. 1	3.44	2.81	2.84	.338	.292	.416	.0187	.0097	.0149
Lub.[1]'41	nf	May 16	3.91	3.53	3.28	.727	.610	.592	.0352	.0316	.0293
		May 26	2.95	4.21	3.46	.588	.582	.584	.0344	.0265	.0400
		May 29	3.93	3.95	3.99	.505	.590	.499	.0449	.0367	.0396
		Average	3.60	3.90	3.58	.607	.594	.558	.0382	.0316	.0363
W.H.[1]'38	f	Dec. 1	2.61	2.59	.....	.612	.377	.....	.0130	.0075	.....
		Dec. 1	2.46	2.59	.....	.639	.385	.....	.0132	.0069	.....
W.H.'38	nf	Dec. 1	2.27	2.68	.....	.604	.428	.....	.0143	.0087	.....
		Dec. 1	2.15	2.43	.....	.683	.402	.....	.0117	.0069	.....
Total number of samples having higher content than corresponding pair mate.....			6	12	.....	16	2	.....	18	.....	.....

Sample	Treatment [2]	Harvest date	Fresh basis (per cent)											
			H_2O			Ca			P			Fe		
			Leaf	Stem	Leaf + stem	Leaf	Stem	Leaf + stem	Leaf	Stem	Leaf + stem	Leaf	Stem	Leaf + stem
C.S.[1]'38	f	Nov. 10	89.57	93.59	.....	.31	.20	....	.082	.037	.....	.0026	.0008	.....
C.S.'38	nf	Nov. 10	89.12	92.96	.....	.34	.25	....	.077	.029	.....	.0023	.0007	.....
		Nov. 11	90.38	93.92	.....	.32	.19	....	.064	.029	.....	.0026	.0008	.....
		Nov. 11	89.59	93.14	.....	.34	.23	....	.065	.027	.....	.0020	.0008	.....
		Nov. 4	89.30	94.70	.....	.33	.20	....	.072	.022	.....	.0026	.0005	.....
		Nov. 10	88.06	92.40	.....	.40	.26	....	.087	.042	.....	.0022	.0007	.....
		Average	89.29	93.42	.....	.35	.23	....	.073	.030	.....	.0023	.0007	.....
C.S.'41	f	Nov. 4	92.85	95.10	.....	.19	.14	....	.041	.030	.....	.0020	.0008	.....
		Nov. 17	92.70	94.70	.....	.19	.17	....	.049	.028	.....	.0014	.0005	.....
		Dec. 1	89.35	91.75	91.95	.24	.20	.18	.079	.040	.058	.0017	.0007	.0012
		Jan. 11	89.60	90.70	89.90	.26	.22	.24	.058 •	.034	.044	.0016	.0009	.0014
		Average	91.12	93.06	.....	.22	.18	....	.057	.033	.....	.0017	.0008	.....
C.S.'41	nf	Dec. 1	92.93	91.95	91.52	.24	.23	.24	.024	.024	.035	.0013	.0008	.0013
Lub.[1]'41	nf	May 16	90.24	92.13	89.78	.38	.28	.34	.071	.048	.061	.0034	.0025	.0030
		May 26	89.73	92.33	90.25	.30	.32	.34	.060	.045	.057	.0035	.0020	.0039
		May 29	90.94	89.12	86.18	.36	.43	.55	.046	.064	.069	.0041	.0040	.0055
		Average	90.30	91.19	88.74	.35	.34	.41	.059	.052	.062	.0037	.0028	.0041
W.H.[1]'38	f	Dec. 1	87.64	91.20	.....	.32	.23	....	.076	.033	.....	.0016	.0007	.....
		Dec. 1	87.02	90.79	.....	.32	.24	....	.083	.035	.....	.0017	.0006	.....
W.H.'38	nf	Dec. 1	88.94	90.07	.....	.25	.27	....	.067	.042	.....	.0016	.0009	.....
		Dec. 1	87.15	90.33	.....	.28	.24	....	.088	.039	.....	.0015	.0007	.....
Total number of samples having higher content than corresponding pair mate			2	16	.....	15	3	....	16	1	.....	18	.....	.....
									1 same					

[1] C.S. means College Station; Lub., Lubbock; W.H., Winter Haven. [2] f means fertilized; nf, not fertilized.

TABLE 3

Mean Content[1] of Ca, P, and Fe of Turnip Greens With Standard Error, Standard Deviation, and Coefficient of Variability

Place grown	Year	Treatment [2]	Number of samples	Plant part	Dry basis (per cent)											
					Ca			P			Fe			H_2O		
					M ± SE	SD	CV	M ± SE	SD	CV	M ± SE	SD	CV	M ± SE	SD	CV
College Station	1938	nf	5	Leaf	3.23±.06	.13	4.10	.682±.018	.040	5.94	.0220± .0017	.0038	17.45	.....	.....	.....
			5	Stem	3.46±.12	.26	7.60	.450±.030	.068	15.16	.0107± .0009	.0020	19.01	.....	.....	.....
College Station	1941	f	4	Leaf	2.49±.09	.18	7.17	.636±.043	.087	13.66	.0199± .0029	.0059	29.45	.....	.....	.....
			4	Stem	2.68±.18	.36	13.38	.497±.053	.105	21.20	.0114± .0020	.0040	35.23	.....	.....	.....
Lubbock	1941	nf	3	Leaf	3.60±.32	.56	15.64	.607±.065	.112	18.52	.0382± .0034	.0058	15.30	.....	.....	.....
		nf	3	Stem	3.90±.20	.34	8.74	.594±.008	.014	2.40	.0316± .0029	.0051	16.14	.....	.....	.....
		nf	3	Leaf + stem	3.58±.21	.37	10.26	.558±.030	.052	9.24	.0363± .0035	.0061	16.71	.....	.....	.....
					Fresh basis (per cent)											
College Station	1938	nf	5	Leaf	.35±.02	.03	9.78	.073±.004	.009	12.76	.00233±.00012	.00027	11.59	89.29± .38	0.84	0.94
			5	Stem	.23±.01	.03	12.39	.030±.003	.008	25.91	.00070±.00006	.00014	20.71	93.42± .40	0.90	0.96
College Station	1941	f	4	Leaf	.22±.02	.04	16.60	.057±.008	.016	28.71	.00170±.00012	.00024	14.35	91.12± .95	1.91	2.10
			4	Stem	.18±.02	.04	20.21	.033±.002	.005	15.11	.00075±.00009	.00019	24.89	93.06±1.08	2.17	2.33
Lubbock	1941	nf	3	Leaf	.35±.02	.04	11.60	.059±.007	.013	21.44	.00368±.00020	.00034	9.26	90.30± .35	0.61	0.67
		nf	3	Stem	.34±.04	.08	22.70	.052±.006	.010	20.02	.00280±.00059	.00102	36.60	91.19±1.04	1.80	1.97
		nf	3	Leaf + stem	.41±.07	.12	30.36	.062±.004	.006	9.97	.00412±.00072	.00125	30.45	88.74±1.28	2.23	2.51

[1] Calculated only for groups consisting of three or more samples. [2] f means fertilized; nf, not fertilized.

TABLE 4

Statistically Significant Differences Between Stems and Leaves of Turnip Greens in Percentage Content of Ca, P, Fe, and H_2O

Sample	Year	Treatment[1]	Number samples each of stems and leaves	Dry basis			Fresh basis			
				Ca	P	Fe	Ca	P	Fe	H_2O
College Station	1938	nf	5	.....	L>S[2]**	L>S**	L>S**	L>S**	L>S**	S>L**
College Station	1941	f	4	.....	.....	L>S**	.....	L>S*	L>S**	.....
Lubbock	1941	....	3	.....	.....	.....	.....	.....	.....	.....
Winter Haven	1938	f	2	.....	L>S**	L>S**	L>S**	L>S**	L>S**	S>L**
Winter Haven	1938	nf	2	.....	L>S**	L>S**	.....	L>S**	L>S**	S>L**

[1] f means fertilized; nf, not fertilized. [2] L means leaf; S, stem; * means significant at the five per cent level, ** at the one per cent level.

TABLE 6

Significant Differences as Shown by Variance Analysis of Turnip Greens Grown at Winter Haven

Comparison	Ca	P	Fe	Ash	Protein	Fat	Fiber	N-free extract	Water
				Dry basis					
Leaf *vs.* stem	....	f**L>S[1] nf**L>S	f*L>S nf**L>S	f**S>L nf**S>L	f**L>S nf**L>S	....	f*S>L	f**L>S nf**L>S	....
f vs. nf	.....	.....	.....	.....	.....	.....	S*f>nf	.....	.....
Interaction	.....	.....	.....	.....	.....	.....	*	.....	.....
				Fresh basis					
Leaf *vs.* stem	f**L>S	f**L>S nf*L>S	f**L>S nf**L>S	f**S>L nf**L>S	f**L>S nf**L>S	....	....	....	f**S>L nf*S>L
f vs. nf	L*f>nf	.....	.....	L**f>nf	.....	.....	.....	.....	.....
Interaction	*	.....	.....	**	.....	.....	.....	.....	.....

[1] L means leaf; S, stem; f means fertilized; nf, not fertilized; * means significant at the five per cent level, ** at the one per cent level.

four places in Mississippi) the iron of the leaf blades (dry basis) was two to three and one-half times that of petiole and midrib, while the calcium content of leaves and stems was almost the same. In contrast, are the findings for calcium and iron in 10 samples of Seven-Tops turnip greens produced the same year in Norfolk, Virginia, by seed from the same source as the Mississippi greens and analyzed in the same laboratory.[3] In every pair of the 10 samples the percentage of calcium in stems exceeded that in leaves by amounts ranging from 0.39 to 1.02 and averaging 0.73. Iron in the stems averaged 0.0116 per cent, in the leaves 0.0159 per cent, or 37 per cent more than the stems. Phosphorus in the leaves of the 50 samples analyzed by the Mississippi Station ranged from 40 to 70 per cent more than in the stems; in this study of Texas greens, phosphorus content in leaves was greater by 15 to 70 per cent than in stems.

A complete record of the results of chemical analysis of the greens from Winter Haven is shown (Table 5), and of variance analysis of data for the same samples (Table 6).

Statistically, highly significant differences between leaves and stems, both fertilized and not fertilized, were found for total ash and protein (nitrogen $\times$ 6.25) on both the dry and the fresh basis; significant differences were found for fiber and highly significant for nitrogen-free extract, dry basis only. Among these samples showing significant differences, the leaves, both fertilized and unfertilized, contained more protein and nitrogen-free extract than did stems. In fiber and in total ash, stems exceeded leaves except that unfertilized leaves were higher in total ash than were corresponding stems.

The literature affords little for comparison with findings in this study. Wang (1936) analyzed each of five vegetables (rape, cabbage, spinach, celery, and coriander) in three parts—lamina, stalk, and central portion. Calcium and iron were found to be most abundant in the lamina, phosphorus in the central portion. Calcium was of moderate amount in the stalks, lowest in central portion; phosphorus and iron were lowest in the stalk.

Sheets (1941) reported for a group of leafy vegetables (turnip greens, collards, mustard, lamb's-quarters, and pokeweed) that iron (dry basis) was two to four times as abundant in leaves as in petioles. Pokeweed leaves were higher also in calcium and phosphorus than the petioles. Maranon[4] (1935) found higher content of calcium and iron in the leaf than petiole of beets but the same of phosphorus (Ca, 2.26 per cent leaf, 0.37 per cent petiole; Fe, 0.19 and 0.10; P, 0.55 and 0.56). He reports for gabi also higher content of calcium in the leaf (1.59 per cent leaf *vs.* 1.00 per cent petiole), but nearly the same content of iron (leaf 0.07 per cent, petiole 0.06 per cent), and more phosphorus in petiole (leaf 0.52 per cent, petiole 0.72 per cent). Fagan,[4] Phillips, and Davies (1943) found more calcium in the lamina than the petiole of kale, but little difference in phosphorus content (Ca, 2.79 and 1.81 per cent leaf and petiole, respectively; P, 0.29 and 0.31 per cent). Comparative data for total ash (dry basis) [Maranon

[3] Personal communication.

[4] Calculated from authors' data given as CaO, P_2O_5, and Fe_2O_3.

TABLE 5

Composition of Four Individual Samples of Leaves and Stems of Turnip Greens Grown at Winter Haven, 1938

Constituent	Treatment [1]	Dry basis		Fresh basis	
		Leaf	Stem	Leaf	Stem
		pct.	*pct.*	*pct.*	*pct.*
Ca	nf	2.27	2.68	0.25	0.27
		2.15	2.43	0.28	0.24
	f	2.61	2.59	0.32	0.23
		2.46	2.59	0.32	0.24
P	nf	0.604	0.428	0.067	0.042
		0.683	0.402	0.088	0.039
	f	0.612	0.377	0.076	0.033
		0.639	0.385	0.083	0.035
Fe	nf	0.0143	0.0087	0.0016	0.0009
		0.0117	0.0069	0.0015	0.0007
	f	0.0130	0.0075	0.0016	0.0007
		0.0132	0.0069	0.0017	0.0006
Total ash	nf	14.58	19.19	1.61	1.90
		13.44	19.28	1.73	1.86
	f	16.04	20.32	1.98	1.79
		15.34	19.24	1.99	1.77
Protein	nf	37.19	20.25	4.11	2.01
		38.19	18.75	4.91	1.81
	f	32.94	18.44	4.07	1.62
		36.94	19.31	4.79	1.78
Fat	nf	4.70	6.35	0.52	0.63
		5.70	2.11	0.73	0.20
	f	5.65	1.89	0.70	0.17
		4.88	2.93	0.63	0.27
Fiber	nf	8.05	8.42	0.89	0.84
		7.34	6.84	0.94	0.66
	f	7.43	10.78	0.92	0.95
		8.66	11.35	1.12	1.04
N-free extract	nf	35.48	45.79	3.93	4.55
		35.33	53.02	4.54	5.14
	f	37.94	48.57	4.69	4.27
		34.18	47.17	4.45	4.35
H_2O	nf	.....	.....	88.94	90.07
		.....	.....	87.15	90.33
	f	.....	.....	87.64	91.20
		.....	.....	87.02	90.79

[1] f means fertilized; nf, not fertilized.

(1935) and Fagan *et al.* (1943)] are as follows: kale 9.75 per cent lamina, 8.72 per cent petiole; beet leaf 22.4 per cent, petiole 21.0 per cent; gabi, 13.5 per cent leaf, 19.2 per cent petiole; turnip greens of this study, 14.0 per cent leaf, 19.2 per cent stems of unfertilized samples and 15.7 per cent leaf and 19.8 per cent stem of fertilized samples. Fiber content of kale, Fagan *et al.* (1943), is considerably higher, 17.6 per cent lamina and 22.3 per cent petiole, than for turnip greens as here reported, 8.0 and 7.7 per cent for fertilized and unfertilized leaves, respectively, and 11.0 and 7.6 per cent for corresponding stems.

Data related to the nutritive value of these greens are presented (Tables 5 and 7) as they may be judged by composition of raw greens, and for certain of the constituents by comparison with the daily allowance for a moderately active grown man as recommended by the Food and Nutrition Board, National Research Council (1943) and Sherman and Lanford (1940). As with samples of leaves plus stems in a previous study by Whitacre *et al.* (1944) one serving (equivalent to 100 grams raw) of these separate leaves and stems would have furnished unimportant amounts of nitrogen-free extract, fat, phosphorus, and protein. Only a portion of the nitrogen-free extract (average 8.15 grams in 100 grams of leaves, 6.09 in 100 grams of stems) is digestible carbohydrate. The fat of the leaves (0.64 gram per 100 grams) would supply less than one-half of one per cent of the energy of a 3,000-calorie diet; the stems only one-half as much as leaves. This is a very meager contribution in comparison with the usual recommendation that fat and carbohydrate should provide from 85 to 90 per cent of the total daily energy. Protein from the stems (1.81 grams per 100 grams) is practically negligible, and that from the leaves (4.5 grams per 100 grams) is unimportant compared with a total daily allowance of 70 grams for an adult man and 80 to 100 grams for adolescents. Phosphorus in leaves and stems together in any sample was not equivalent to as much as five per cent of the allowance.

Average fiber content of 1.00 per cent in both leaves and stems is of similar amount to that of other leafy vegetables which are regarded as good sources of bulk in the diet. Total ash content ranging from 1.61 to 1.99 grams per 100 grams in the leaves and 1.77 to 1.90 in the stems (fresh basis) is comparable with that of other foods prized for a mixture of minerals. The calcium, phosphorus, and iron together in these greens constitute approximately 20 per cent of the total ash of the leaves and 15 per cent of that in the stems. The remainder of the total ash (80 per cent for leaves, 85 per cent for stems) indicates that both plant parts are good sources of other minerals essential to nutrition, and in this respect the stems have a slight advantage, assuming equal availability.

The samples selected for inclusion (Table 7) show the contributions of calcium, phosphorus, and iron which would come from the greens with the highest and those with the lowest proportion of leaves, by the greens whose stems contained the minimum and others the maximum values found for calcium, phosphorus, and iron, respectively. One serving of these turnip greens would supply from 22 to 50 per cent of the recommended daily allowance of calcium, the range for the stem portion of one serving being 6.2 to 27.5 per cent, and of the leaves from 15.0 to 23.8 per cent. Leaves

TABLE 7

Contribution of Ca, P, and Fe to Diet From Leaves and Stems of One Serving[1] of Turnip Greens

Sample			Weight (gm. in 100 gm. greens)	Content, fresh basis			Gm. from leaf and stem portions of 100 gm. greens (leaf + stems)			Proportion of allowance[2] in samples		
				Ca	P	Fe	Ca	P	Fe	Ca	P	Fe
			pct.	*pct.*	*pct.*	*pct.*				*pct.*	*pct.*	*pct.*
With highest proportion of leaves (nf)	Winter Haven Nov. 1, '38	Leaf	75.55	.25	.067	.0016	.19	.051	.00121	23.8	3.9	10.1
		Stem	24.45	.27	.042	.0009	.07	.010	.00022	8.8	0.8	1.8
		Total	.....	....	.....	.....	....	.....	.....	32.6	4.7	11.9
With lowest proportion of leaves (also lowest P in stems) (nf)	College Station Nov. 4, '38	Leaf	48.50	.33	.072	.0026	.16	.035	.00126	20.0	2.6	10.5
		Stem	51.50	.20	.022	.0005	.10	.011	.00025	12.5	0.8	2.1
		Total	.....	....	.....	.....	....	.....	.....	32.5	3.4	12.6
With highest content of Ca in stem (also highest Fe and P) (nf)	Lubbock May 29, '41	Leaf	49.12	.36	.046	.0041	.18	.022	.00201	22.5	1.7	16.8
		Stem	50.88	.43	.064	.0040	.22	.032	.00204	27.5	2.4	17.0
		Total	.....	....	.....	.....	....	.....	.....	50.0	4.1	33.8
With lowest content of Ca in stem (f)	College Station Nov. 4, '41	Leaf	66.67	.19	.041	.0020	.13	.027	.00133	16.2	2.0	11.1
		Stem	33.33	.14	.030	.0008	.05	.010	.00027	6.2	0.8	2.2
		Total	.....	....	.....	.....	....	.....	.....	22.4	2.8	13.3
With lowest content of iron in stem (f)	College Station Nov. 17, '41	Leaf	61.89	.19	.049	.0014	.12	.030	.00087	15.0	2.3	7.2
		Stem	38.11	.17	.028	.0005	.06	.011	.00019	7.5	0.8	1.6
		Total	.....	....	.....	.....	....	.....	.....	22.5	3.1	8.8
Average of all 18 samples		Leaf	63.33	.30	.066	.0022	.19	.042	.00139	23.8	3.2	11.6
		Stem	36.67	.24	.036	.0011	.09	.013	.00040	11.2	1.0	3.3
		Total	.....	....	.....	.....	....	.....	.....	35.0	4.2	14.9

[1] 100 grams raw, about one-half cup cooked. Assume all liquor eaten and nutrients available to human body. [2] Recommended daily allowance: Ca 0.8 gm., P 1.32 gm., Fe 0.012 gm.

may therefore be regarded as an excellent source and stems a fair to good source of calcium. Contribution of iron from leaves ranged from 7.2 to 16.8 per cent of the allowance, while stems furnished approximately one-fifth as much, with exception of one sample whose stems, practically speaking, were as high in iron as the leaves. Considering that iron in the well-selected diet must come from a number of sources, a contribution of 10 per cent of the allowance should be regarded as valuable and smaller amounts not disregarded. In one sample the leaves supplied less phosphorus than the stems, but among others (Table 7) the leaves furnished two to four times as much phosphorus as stems. Content of phosphorus in leaves and stems together, however, is too low to give turnip greens consideration as a source of this mineral. With content of fiber the same in leaf and stem, it seems justifiable to assume equally good utilization of mineral from the two plant parts. The character of fiber might be sufficiently different in stems and leaves to influence nutritive value, however, and fiber may exert different effects upon the several nutrients while in the digestive tract.

The reason commonly given for discarding stems of turnip greens—that they are tough—has been shown to be invalid in a previous study by Whitacre *et al.* (1944). The mechanical shearing strength of both leaves and stems increased at the same rate. Also stems and leaves were usually made tender by cooking in the same length of time. The "stringiness" of mature turnip greens stems after cooking is objectionable to some persons, but as with other foods, usually a liking for them will be developed by eating them. In the interest of the highest conservation of certain nutritive values—fiber, total ash, and calcium and, to a less extent, iron—in turnip greens it seems wise on the basis of chemical composition to encourage the use of stems as well as leaves. Final answer as to their relative nutritive value must await the results of experiment with human subjects.

SUMMARY AND CONCLUSIONS

A comparison has been made between the leaves and stems of turnip greens regarding content of water, calcium, phosphorus, and iron, using 18 pairs of the two plant parts and five samples of leaves plus stems. In some of these samples the contents of fat, nitrogen, nitrogen-free extract, total ash, and fiber were also determined. The samples represented the crops of two different years and the greens grown in three localities in Texas.

Leaf portion of the greens comprised an average of 63.3 per cent and stems 36.7 per cent of the total weight of the greens in the fresh state after washing and eliminating surface water. In general, leaves exceeded stems in content of phosphorus and iron, both dry basis and fresh basis. With calcium, 12 of 18 stem samples (dry basis) exceeded their mate sample of leaves, while on the fresh basis stem-leaf relation was reversed, 15 leaf samples containing more of this mineral. In all statistically significant differences between leaves and stems, leaves were higher in calcium, phosphorus, iron, protein, and nitrogen-free extract; stems were higher in water, fiber, and total ash, with the single exception that leaves of unfertilized greens exceeded the stems in total ash content. In one group of

samples no significant differences were found; calcium content of leaf and stem portions was practically the same, phosphorus slightly less in the stems than leaves, iron 32 per cent more in leaf than stem.

Irrespective of fertilizer treatment and of place and year grown, the minimum and maximum values among individual samples on the fresh basis were for leaves, 0.19 and 0.40 per cent calcium, 0.024 and 0.088 per cent phosphorus, 0.0013 and 0.0041 per cent iron; for stems, 0.14 and 0.43 per cent calcium, 0.002 and 0.064 per cent phosphorus, and 0.0005 and 0.0040 per cent iron. Calcium, phosphorus, and iron together constituted on the average 20 per cent of the total ash of the leaves, 15 per cent of the ash of the stems. Hence stems with 85 per cent of the total ash (*vs.* 80 per cent in leaves) were a slightly better source of the other essential minerals, assuming equal availability from stems and leaves. Total ash ranging from 1.61 to 1.99 per cent in the leaves, from 1.77 to 1.90 per cent in stems (fresh basis) places these greens among the foods valued for a mixture of minerals. One serving (100 grams raw) of the stem portion of these greens would give from 6.2 to 27.5 per cent of the recommended daily allowance of calcium; the leaves from 15.0 to 23.8 per cent. The contribution of iron from the leaves ranged from 7.2 to 16.8 per cent of the allowance, while stems furnished approximately one-fifth as much. Fiber content of 1.0 per cent in both stems and leaves, fresh basis, makes turnip greens valuable for bulk in the diet. Turnip greens appear to be of little consequence for phosphorus, protein, nitrogen-free extract, and fat.

Compared with current recommended daily allowances, leaves of turnip greens may be regarded as an excellent, and stems a fair to good, source of calcium; for iron, leaves may be rated good, stems fair to poor. As shown by data reported here and elsewhere, however, stems may resemble leaves in composition. Findings in this study support the recommendation that stems as well as leaves of turnip greens be eaten as a means of conserving constituents of importance nutritionally—fiber, total ash, calcium, and iron.

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A COMPARATIVE STUDY OF SOY FLOURS FOR USE IN BAKING
I. COMPOSITION AND PROPERTIES OF SOY FLOURS

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A number of problems arise when soy flours are considered for use in appreciable amounts in baking. One of these is the fact that soy flours differ in chemical composition and in physical properties which are important in determining baking quality. The purpose of this study has been to note some of these differences. Not included in this investigation are certain questions in connection with the use of soy flours, such as the shortening value of the fat as it occurs in soy flours, the importance of their lecithin content, and of the quality and quantity of the protein they contain.

THE PROBLEM

Types and Composition: Soy flours are usually classified according to the amount of fat they contain. Precise methods of classification vary, however, as do the terms used to describe them. As a rule, the flours are considered to be of three types. The first of these is the minimum-fat or no-fat type, containing one per cent fat or less. This is sometimes referred to as high-protein flour. The second type is the low-fat flour (occasionally called the medium-fat flour), containing approximately four to eight per cent fat, and the third type is the high-fat or full-fat flour which contains 18 to 24 per cent fat.

The composition of a number of soy flours of different types is described by Bailey, Capen, and Le Clerc (1935). It is clear from their figures that numerous constituents vary with the fat content. Such variables include protein, minerals,fiber, and lecithin, all of which no doubt bear an important relationship to the baking quality of the samples. The question has been raised as to whether this lack of standardization of soy flours may not be a major problem in using them, necessitating adjustments in the formulas and methods employed according to the type of soy flour.

Weight per Unit Volume: Grewe (1932a, 1932b, and 1932c) found considerable variation in the weight of a given volume of different wheat flours and drew attention to possible factors affecting it. It is to be expected that soy flours also differ in this respect, although no information of this nature seems to be available.

Capacity for Water Absorption: Batters and doughs containing appreciable amounts of soy flour appear to be generally stiffer than those prepared entirely from wheat flour, and this difference may no doubt be explained on the basis of the greater absorption of water by the soy flour. Such capacity has been noted by Bell (1933) who found that more water

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was required in making bread prepared partly from soy flour. Statements are also made by Dietz (1933), Horvath (1931), and Levenson (1942) to the effect that soy flours have high water-absorption properties. Bailey, Capen, and Le Clerc (1935), however, found that more water was required only when the soy flour was the minimum-fat type. According to Dietz (1933), this feature of greater water-absorption capacity is utilized in some instances for the purpose of tightening up weak and slack doughs.

It is due to their large water-absorption capacity that additional liquid is required in preparing products from soy flours. No information is available, however, as to how the different types of soy flours compare on this basis, and therefore the extent to which liquid must be increased according to the type used.

Degree of Granulation: One of the characteristics of wheat flour that varies with the type is the degree of granulation or size of particle, and Grewe (1932c) has indicated that this fact bears an important relation to the weight per unit volume of the sample. She found that the coarser wheat flours weighed consistently more per cup than the finer ones. Whether there are similar differences between soy flours needs to be determined.

Color: The color of soy flour is important because if it is not satisfactory, it may impart to the baked product an unattractive dull effect. A light cream color is desirable, but occasionally the flour shows a tinge of green and, as a result, the products prepared from it are slightly off-color. According to Eastman (1944), the colors of different samples vary from almost white to brown.

Flavor: One of the factors limiting the proportion of soy flour that can be used in baking is the flavor it imparts to the products, and numerous methods have been suggested for treating the beans at different stages in the preparation of the flour, in an attempt to remove the "beany" flavor which is characteristic and unpleasant. These methods have been described in some detail by Bailey, Capen, and Le Clerc (1935); and according to the soy flours available at present, it seems they are used with varying success. The special value of ethanol extraction in its effect on both flavor and color has been indicated by Beckel and Smith (1944).

Keeping Quality: Frequent comments have been made to indicate that soy flours, particularly the high-fat type, become rancid readily. Such opinions have also been expressed by soy flour companies in personal communication with the authors, explaining in part the reason for promoting minimum-fat or low-fat rather than high-fat soy flours.

Some workers, however, indicate that recent improvements in the method of manufacturing soy flours from the standpoint of flavor also improve their keeping quality. The improvement mentioned by McBride (1940) as having particular value in this respect is treatment by steam, such treatment serving primarily to inactivate enzymes which otherwise hasten rancidity. Weiss (1940) has gone so far as to claim that full-fat soy flour has extraordinary keeping qualities.

Questionnaire to Manufacturers and Distributors: For the purpose of this study it seemed desirable to know what types of soy flour are being distributed and the impressions of manufacturers and distributors regard-

ing the problems with which they are confronted in promoting these products. A questionnaire was sent, therefore, to 36 different companies, 20 of which replied. According to these replies, the market for soy flours ranges all the way from small to excellent, and opinions differ as to the type of soy flour that should be promoted.

EXPERIMENTAL PROCEDURE

Methods: Eighteen samples of soy flour were obtained from different parts of the United States. Preliminary inspection indicated that marked differences existed between the three types of flour and also between the samples within each group. They varied considerably in color and in their weight per cup. It was noted also, that the minimum-fat flours were powdery, whereas those in the high-fat group had a tendency to pack. Batters incorporating different soy flours were found to vary in consistency from very stiff when prepared from minimum-fat flour, to medium or thin when the same weight of high-fat flour was used.

Fat and Moisture Content: The soy flours were analyzed for fat and moisture content by the official methods of the Association of Official Agricultural Chemists (1940). The results are the average of three determinations in all cases.

Weight per Cup: A comparison of the weights of various soy flours was made according to the methods described by Grewe (1932a). Ten weighings of each sample were made by each of three persons, and from these figures the averages were computed.

Capacity for Water Absorption: Various methods for determining the water-absorption capacity for flours are reported in the literature, but the one used in this study is an adaptation of an unpublished procedure developed previously in this laboratory by Woodruff and Engdahl.

Five grams of soy flour were weighed into a 50-ml. graduated centrifuge tube, and 25 ml. of distilled water was added from a burette. The flour and water were thoroughly mixed and an additional five ml. of water was added to wash down the sides of the tube and the stirring rod. The suspension was allowed to stand at room temperature for one hour, then was centrifuged. The total volume of the flour and water and the volume of the flour alone were then noted, the difference between these readings giving the excess of water in the suspension. The difference between the original volume of water added to the flour (30 ml.) and the volume of water not absorbed is the amount of water absorbed by the five grams of flour. The results are reported as milliliters of water absorbed by 100 grams of soy flour and are the average of at least five determinations in all cases.

Granulation: Since granulation of a flour is frequently described in terms of the proportion of the sample which passes through a sieve of a given mesh, this method of determining granulation was attempted. It was found, however, that the high-fat soy flour clogged the sieve in such a manner that the method was impracticable. Instead, microscopic measurement of the size of flour particle was used as a means of indicating the degree of granulation.

An adaptation of the microscopic method described by Hildebrand, Ferrari, Borchardt, and Anker (1942) was employed. A small amount of flour was placed on a microscopic slide and a few drops of dilute collodion were added. The particles of flour were separated by stirring vigorously with a pointed glass rod, then the collodion was allowed to dry. No cover glasses were used. The flour particles were observed under a magnification of 450 times.

The particles were measured in microns with an ocular micrometer previously calibrated by means of a stage micrometer. For each particle, the measurement was made at the largest diameter, the ocular being turned as necessary to make this possible. Particles were counted beginning at one corner of the slide and the slide was moved in such a manner as to prevent looking at the same portion of it twice. Measurements were continued until 300 particles had been included, this large number was chosen because the measurements varied over a wide range. The results are the average of 300 measurements in all cases.

Color: The eighteen samples of soy flour were matched with the colors in the Maerz and Paul (1930) "Dictionary of Color." This procedure was only partially satisfactory, since the plates were not found to include the exact duplicates of some of the samples. In a further attempt to learn whether there is a relationship between the color and the type of soy flour, the 18 samples were divided into three groups, designated as light, medium, and dark, respectively. The number of high-fat, low-fat, and minimum-fat soy flours which had been placed in each group was afterwards noted.

Keeping Quality: A controlled study of the keeping quality of soy flours was not included. For the purposes of this investigation, the samples were all stored under ordinary laboratory conditions in closed containers for approximately six months.

RESULTS AND DISCUSSION

The results (Table 1) show that percentages of fat (dry basis) according to the type of soy flour were found to be as follows: minimum-fat, 0.57 to 1.01; low-fat, 3.50 to 8.00; high-fat, 20.37 to 23.77. The moisture content of the flours varied from 4.67 to 7.42 per cent, with a tendency for the minimum-fat flours to be higher in moisture than the high-fat samples, and for the low-fat ones to be intermediate.

The minimum-fat soy flours weighed approximately from 104 to 125 grams per cup, and four out of five low-fat flours fell within the range of 73 to 100 grams per cup. When the two samples showing exceptionally high weights are excluded, the high-fat soy flours are found to range approximately from 55 to 59 grams per cup. It is thus evident that the minimum-fat soy flours for the most part weigh about twice as much per cup as the high-fat samples, with the low-fat ones intermediate. The weights for samples No. 8, 9, 17, and 18 are not in good agreement with the other members of their respective groups, the values in all these cases being higher. In Samples 8 and 18, this may be explained possibly by the fact that these two flours are rather uneven and seem to contain appreciable amounts of bran-like material. No explanation is suggested for the higher weights of Samples 9 and 17.

The amount of water absorbed per 100 grams of soy flour has been found to decrease as the fat content increases. The minimum-fat, low-fat, and high-fat flours absorbed, respectively, 250 to 280, 240 to 250, and 170 to 230 milliliters of water per 100 grams of flour. This difference is especially interesting in view of the fact that batters prepared from minimum-fat soy flours have been found to require considerably more liquid than those prepared from the other types of soy flour.

TABLE 1

A Comparison of 18 Samples of Soy Flour

Type of flour	Sample No.	Moisture content	Fat content		Weight per cup of flour	Water absorbed by 100 gm. flour	Average diameter of flour particle	Color	
			Wet basis	Dry basis				Plate No.[1]	Color group
		pct.	*pct.*	*pct.*	*gm.*	*ml.*	*microns*		
Minimum-fat	1	7.32	0.94	1.01	118.9	250	31.6	9B1	Light
	2	7.04	0.53	0.57	104.2	280	29.3	9B1	Light
	3	6.89	0.91	0.98	124.5	260	27.0	9C2	Medium
	4	7.42	0.87	0.94	125.0	260	32.0	9C2	Medium
Low-fat	5	5.43	6.17	6.52	73.4	240	34.2	9B1	Medium
	6	6.56	3.37	3.50	77.1	250	37.6	9B1	Light
	7	5.79	7.54	8.00	72.9	240	36.7	9C2	Medium
	8	6.37	5.40	5.77	108.8	240	32.9	9B2	Dark
	9	6.57	5.47	5.86	98.6	240	29.7	9C2	Medium
High-fat	10	4.88	22.28	23.42	55.4	180	52.0	9B1	Medium
	11	4.75	22.64	23.77	56.2	180	43.8	9D2	Dark
	12	5.19	22.53	23.76	57.5	170	47.7	9B1	Medium
	13	4.90	22.26	23.41	58.0	180	50.1	9C2	Dark
	14	5.39	20.39	21.55	59.1	230	54.8	9C1	Dark
	15	4.67	22.11	23.19	59.3	180	54.3	9C2	Medium
	16	5.86	21.31	22.64	58.9	230	50.6	9D1	Dark
	17	5.61	21.95	23.25	83.2	180	44.0	9D2	Dark
	18	5.16	19.32	20.37	105.3	180	47.9	10C2	Dark

[1] The plate number is that of the Maerz and Paul "Dictionary of Color" used in matching the sample.

The average diameter of soy-flour particle ranged from 27 to 54.8 microns, with a tendency for the minimum-fat flour particles to be small, those of the high-fat flours to be large, and those of the low-fat samples to be intermediate in size.

The colors of soy flours as determined by matching with the Maerz and Paul "Dictionary of Color" did not indicate any particular relationship between the color of the sample and its fat content. It seems that this fact is due in part at least to the difficulty encountered in finding perfect matches for a number of the samples. Dividing the flours into three groups—light, medium, and dark—showed better the expected relationship. Of the seven samples in the dark group, six were of the high-fat type, the three other flours of this type occurring in the medium group. No clear-cut grouping of the minimum-fat and low-fat soy flours was indicated on the basis of color. These observations were in accordance with previous impressions obtained in working with the flour, when it was felt that the high-fat samples in general were considerably darker than the others.

During the six months' storage period in which the soy flours were held for this investigation, none of the samples developed an apparent rancidity as judged by their flavor or odor. No difference was found between the types of soy flour on the basis of keeping quality.

CONCLUSIONS

According to the 18 samples included in this investigation, soy flours vary considerably in composition and certain properties which are important in determining baking quality. Clear-cut differences according to type of flour were not found, but a tendency was indicated for moisture content, weight per unit volume, and water-absorption capacity to decrease with increase in fat content. The size of flour particle, on the other hand, tends to increase with increase in fat content. The high-fat soy flours seem inclined to be somewhat darker in color than those of either minimum-fat or low-fat type.

SUMMARY

Eighteen samples of soy flour from different parts of the United States, including minimum-fat, low-fat, and high-fat types, were compared as to certain characteristics important in determining baking quality. Clear-cut differences according to the type of soy flour were not found, but a tendency was indicated for moisture content, weight per unit volume, and water-absorption capacity to decrease as the fat content increases. The size of flour particle, on the other hand, tends to increase with increase in fat content. The high-fat flours seem inclined to be somewhat darker in color than those of either the minimum-fat or low-fat type.

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A COMPARATIVE STUDY OF SOY FLOURS FOR USE IN BAKING
II. EFFECT ON QUALITY OF MUFFINS AND CAKES

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This study was undertaken to determine the effect on palatability when soy flour is used in muffins and in plain cake, and to determine the relative merits of a number of samples of soy flours of different fat content.

So little has been done to date in well-controlled studies concerning the use of soy flour in baking, that directions are far from well established. The popular recommendation is that as much as two tablespoons of soy flour can replace two tablespoons of wheat flour in each cup of flour used for baking. According to the data obtained by Faulkner and Simpson (1946) this proportion by measure equals approximately the following by weight: seven per cent high-fat soy flour, nine per cent low-fat soy flour, and 13 per cent minimum-fat soy flour. It would seem that such substitutions as this cannot be expected to meet with more than partial success, when they are made regardless of the type of soy flour and of the kind of product prepared from it.

More work of an experimental nature has been done with soy flour in bread than in any other baked product, and the recommended proportions vary considerably. Bailey, Capen, and Le Clerc (1935) state that bread can be made with 10 per cent soy flour without changing the regular baking method and that by making necessary adjustments, bread can be made satisfactorily with as much as 30 per cent soy flour. They also state that this high proportion can be used in making cakes and other baked products. Le Clerc and Bailey (1936) found that an occasional angel food cake made with soy flour and wheat flour was superior in tenderness and in some other ways, although they do not recommend this mixture in general for making angel food cake. The most important factors limiting the proportion of soy flour that can be used for general baking purposes would seem to be its characteristic flavor and the fact that it has no gluten and little if any starch.

EXPERIMENTAL PROCEDURE

This investigation was conducted concurrently with "A Comparative Study of Soy Flours for Use in Home Baking. I. Composition and Properties of Soy Flours," by Faulkner and Simpson (1946), and the same 18 samples of soy flour were used for both studies.

Muffins and plain shortened cakes incorporating the soy flours were used as a basis for judging the baking quality of the various samples. The largest proportions of soy flour that could be used in the preparation of acceptable muffins and cakes were ascertained in preliminary experiments and approximately these amounts were used in the investigation. Since

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proportions of ingredients for household purposes are usually determined on the basis of measure, this procedure was used in developing formulas for the first series of muffins, these measures being converted into corresponding weights for laboratory use. It was realized, however, from the weights per unit volume of the flours as determined by Faulkner and Simpson (1946) that when equal measures of the three types of soy flour were used, quite different weights were employed. Hence, a second series of muffins was prepared in which 26.2 per cent of the total flour by weight was soy flour throughout. One series of cakes was prepared in which soy flour constituted 15 per cent of the total flour by weight. The ingredients for the muffins were mixed by hand, but those for the cakes were combined in an electric mixer; the formulas for both series of muffins and for the cakes are given (Table 1).

TABLE 1

Formulas for Muffins and Cakes

Ingredient	Muffins				Cakes[2]
	Series I[1]			Series II[2]	All types of soy flour[4]
	Minimum-fat soy flour[4]	Low-fat soy flour[4]	High-fat soy flour[4]	All types of soy flour[4]	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Soy flour	58.5	40.0	32.0	58.5	18.75
Wheat flour [5]	165.0	165.0	165.0	165.0	106.25
Baking powder	16.4	16.4	16.4	16.4	5.20
Salt	3.0	3.0	3.0	3.0	2.00
Shortening	50.0	50.0	50.0	50.0	54.00
Sugar	50.0	50.0	50.0	50.0	42.00
Egg	48.0	48.0	48.0	48.0	67.00
Milk	325.0	244.0	244.0	325.0[6]	99.00

[1] Amounts based on proportion by measure (approximately four tablespoons of soy flour to 12 tablespoons of wheat flour in all cases). [2] In all cases, 26.2 per cent of the total flour by weight was soy flour. [3] In all cases, 15.0 per cent of the total flour by weight was soy flour. [4] For analysis of these soy flours, see Faulkner and Simpson (1946). [5] All purpose flour for muffins and cake flour for cakes. [6] For high-fat, soy-flour muffins 284 grams of milk were used.

The index to volume measurements of the baked products in muffin Series II and in the cake series were made by means of the polar planimeter. The products were also rated subjectively by a panel of 10 judges, the number of judges varying from five to nine, with an average of seven. The score cards for muffins and for cakes in U. S. Dept. Agr. Farmers' Bulletin No. 1775 (1937) were modified for use in this investigation in which flavor of soy flour was considered especially important, and in addition, judges were requested to give a general rating of each product. The figures for the general rating are those used in the statistical analysis of the results.

A limited study was made to determine the effect of the addition of soy flour to angel food cakes. The three types of soy flour were used to replace part of the wheat flour in amounts from two to 30 per cent. The cakes were judged subjectively and measurements were made of compressibility and of index to volume. This study was undertaken as a problem by a senior student and while the results are not extensive enough to warrant

detailed presentation or analysis, they are mentioned as an interesting contribution.

RESULTS AND DISCUSSION

Examination of the index to volume measurements of the baked products in muffin Series II and in the cake series (Table 2) indicates that there is little difference in volume on the basis of type of soy flour used. Statistical analysis indicates that the differences are not significant in any case.

TABLE 2

Index to Volume Measurements[1] of Muffin Series II and of Cakes

Product	Type of flour used	Number of samples measured	Mean surface area and standard deviation of mean	Difference between means and standard error of difference[2]		
				High-fat flour as basis for comparison	Low-fat flour as basis for comparison	Minimum-fat flour as basis for comparison
			sq. in.			
Muffins—Series II (26.2 per cent by weight of total flour as as soy flour)	High-fat	144	3.45±.028	.....	.13±.945	.04±.043
	Low-fat	90	3.32±.036	.13±.045	.....	.09±.049
	Minimum-fat	71	3.41±.034	.04±.043	.09±.049	.....
Cakes (15 per cent by weight of total flour as soy flour)	High-fat	24	10.69±.153	.....	.46±.205	.26±.179
	Low-fat	12	11.15±.136	.46±.205	.....	.20±.165
	Minimum-fat	11	10.95±.093	.26±.179	.20±.165	.....

[1] Index to volume as measured by polar planimeter, in terms of surface area of cross section of the product. [2] None of these differences are statistically significant.

Results of the subjective ratings of the muffins and cakes (Table 3) are difficult to interpret. According to the mean scores of these ratings, the muffins and cakes prepared from high-fat, minimum-fat, and low-fat soy flours ranked first, second, and third, respectively, in order of preference. However, when the difference between means and standard errors of the difference of the three types of soy flour are considered, it is evident that the results are not statistically significant in any case.

It is interesting to note that according to the standard deviation of the mean scores, the judges were more consistent in their ratings of the high-fat, soy-flour products than in their ratings of the other types. It should be noted also, that the mean scores for all products fall within the group considered "fair" (none below 3.0 nor above 3.9) and the differences therefore are small. It should be reported that it was the impression of those involved in this study that the judges were much more enthusiastic about the muffins prepared from soy flour than the rating of fair would indicate. Comments were repeatedly made upon their attractiveness and good eating quality. This discrepancy provides evidence of the difficulty encountered in evaluating products on subjective bases.

A study of the distribution of scores among the various samples of soy flour (Table 3) makes it evident, as expected, that a large proportion of soy flours gave products which rated fair, particularly in the preparation of cakes. Except for one sample of minimum-fat soy flour used in muffin

TABLE 3

Subjective Ratings of Soy Flour Muffins and Cakes

Product	Type of flour	Mean score[1] and standard deviation of mean	Difference between mean scores and standard error of difference [2]			Distribution of scores [3]					
						Poor		Fair		Good	
			High-fat flour as basis for comparison	Low-fat flour as basis for comparison	Minimum-fat-flour as basis for comparison	Number flours rated	Per cent flours rated	Number flours rated	Per cent flours rated	Number flours rated	Per cent flours rated
Muffins—Series I (4 tablespoons soy flour to 12 tablespoons wheat flour)	High-fat	3.7±.12	.....	.3±.25	.2±.29	....	.....	6	75.0	2	25.0
	Low-fat	3.4±.22	.3±.25	.....	.1±.34	1	20.0	4	80.0	....	.....
	Minimum-fat	3.5±.26	.2±.29	.1±.34	.....	1	25.0	2	50.0	1	25.0
	Total scores	.....	.....	.....	.....	2	11.7	12	70.6	3	17.6
Muffins—Series II (26.2 per cent by weight of total flour as soy flour)	High-fat	3.6±.18	.....	.4±.30	.3±.31	1	12.5	6	75.0	1	12.5
	Low-fat	3.2±.25	.4±.30	.....	.1±.36	2	40.0	3	60.0	....	.....
	Minimum-fat	3.3±.26	.3±.31	.1±.36	.....	1	33.3	2	66.6	....	.....
	Total scores	.....	.....	.....	.....	4	25.0	11	68.7	1	6.2
Cakes (15 per cent by weight of total flour as soy flour)	High-fat	3.7±.12	.....	.3±.22	.1±.20	....	.....	7	87.7	1	12.5
	Low-fat	3.4±.18	.3±.22	.....	.2±.24	1	20.0	4	80.0	....	.....
	Minimum-fat	3.6±.16	.1±.20	.2±.24	.....	....	.....	4	100.0	....	.....
	Total scores	.....	.....	.....	.....	1	5.8	15	88.2	1	5.8

[1] Out of a total score of 5. [2] None of these differences is significant. [3] Rating scale was as follows: very poor, 1; poor, 2; fair, 3; good, 4; very good, 5.

Series I, products prepared from high-fat soy flour are the only ones rated "good." A larger percentage of the total number of flours gave products rated "poor" in muffin Series II than in muffin Series I.

In the limited study of angel food cakes it seemed, as might be expected, that the minimum-fat soy flours were the most satisfactory. Amounts as high as 15 per cent by weight of this type of soy flour were incorporated without seriously decreasing the acceptability of the cakes, whereas only five per cent and two per cent of the low-fat and high-fat flours, respectively, could be used successfully. As the percentage of these two flours in the cakes was increased, the tenderness and volume decreased, the texture and grain became increasingly inferior, and the characteristic flavor and color of the soy flour became more pronounced.

CONCLUSIONS

With the formulas used in this investigation, definite superiority was not found for any one type of soy flour for use in preparing muffins or plain, shortened cake. This cannot be interpreted, however, as indicating that the three types of soy flour are interchangeable in baking, since the formulas used in this study are adjusted to a considerable extent with a view to obtaining good products in all cases. Statistical significance was not found between the differences in either cakes or muffins prepared from the different flours when compared on the basis of either volume measurement or subjective rating. Some degree of preference seems to have been found, however, for the use of high-fat soy flour in the preparation of muffins and possibly of cakes. In the limited study of angel food cake, it was found that larger amounts of minimum-fat soy flour than of either of the other types can be used without detrimental effect on the flavor and other qualities of this product.

SUMMARY

Eighteen samples of soy flour were compared as to baking quality by using them in the preparation of muffins and plain, shortened cakes and of angel food cakes in a limited study. The baked products were rated subjectively and index to volume measurements was made by the polar planimeter.

With the formulas used in this study, differences in quality of the products according to the type of soy flour were not found to be statistically significant. However, some degree of preference seems to have been found for the use of high-fat soy flour in the preparation of muffins and possibly of cakes. In a limited study of angel food cake, it was found that larger amounts of minimum-fat soy flour than of either of the other types can be used without detrimental effect on the flavor or other qualities of the product.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the 10 members of the staff of the Department of Home Economics who served as the panel of judges for rating subjectively the muffins and cakes prepared in this study; to Dr. Frances Van Duyne for her help in the statistical analysis of the data; to Martha Wheeler for contributing the study of angel food

cakes; and to Royene Frantz and Lucille Powers for their laboratory assistance throughout the investigation.

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ALBERT CLAYTON HUNTER

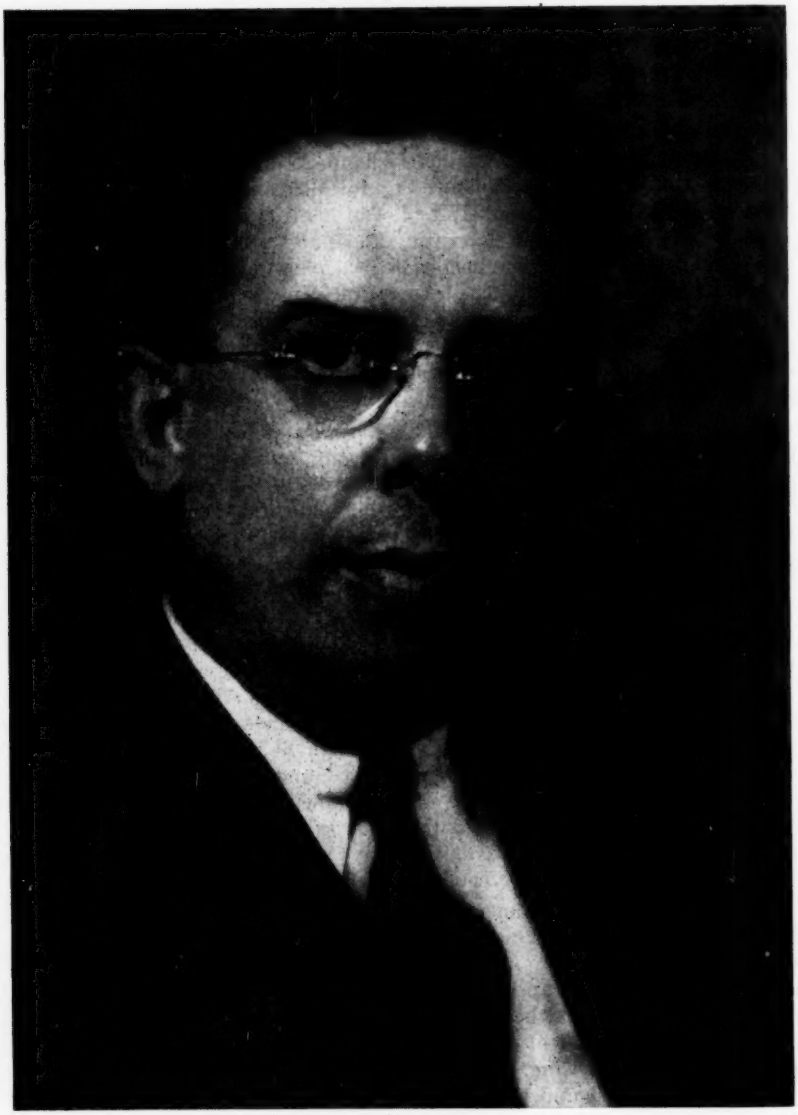
The sudden death of Albert C. Hunter on April 13, 1946, deprived *Food Research* of a valued member of its group of editorial associates. Both the fields of food research and food regulation lost an honored worker and a stimulating leader.

He was born in the state of Rhode Island, received his B.S. degree from Rhode Island State College, followed by his M.S. and Ph.D. in bacteriology from Brown University. In 1918 he began his lifelong association with the Bureau of Chemistry and later the Food and Drug Administration in enforcement problems in the bacteriological phase of food control. During this long association of 28 years, Dr. Hunter made outstanding contributions both to the scientific aspects of food problems and to better sanitary controls throughout the food industry. In his earlier work, he was closely associated with the tremendously difficult and important problems involved in the canning of salmon.

His fine background of scientific training and his firsthand studies of conditions surrounding the handling and canning of salmon, made him an outstanding expert in the judgment of the quality of this processed food. The earlier records of notices of judgment under the Food and Drugs Act of 1906 are filled with court records of successful court action against decomposed salmon, in which cases the evidence was based primarily on Dr. Hunter's knowledge, judgment, and experience in this particular field. He was an outstanding expert witness. Backed by his scientific training, his statements were always based on facts and not conjecture. He was able to withstand successfully the most severe type of cross-examination. A closer study of these court decisions emphasizes the remarkable fact that it is primarily his expert testimony which invariably outweighed the testimony of defense witnesses. Even though he appeared frequently in court cases of that type, he was well liked and highly respected in the salmon-canning industry because of his eminent fairness in dealing with each problem as it arose. Not only in the salmon field, but in the entire sea-food field, his influence was felt—always urging the production of better foods under proper sanitary conditions.

Notwithstanding the numerous and arduous problems which Dr. Hunter faced in the food-control field, he found the opportunity to make many important contributions on the scientific side of food work. At the end of this article there is appended a bibliography of publications of which Dr. Hunter was either the author or coauthor. This list is certainly impressive and indicative of the broad vision, resources, and training of the man. It is not surprising, therefore, that the Association of Official Agricultural Chemists should have called upon Dr. Hunter to serve as its first general referee on microbiological methods, which appointment Dr. Hunter first received in 1933 and held up to the time of his death. The chapter in the A.O.A.C. "Methods of Analysis" entitled "Microbiological Methods" is a monument to the organizing ability of Dr. Hunter.

He was a coauthor with Dr. Charles Thom of "Hygienic Fundamentals of Food Handling," which was published in 1925 by the Williams and



ALBERT CLAYTON HUNTER
1893-1946

Wilkins Company. This book, although 20 years old, is still regarded as a basic and fundamental publication in this field.

His last presentation of a scientific paper was at the March, 1946, convention of the Institute of Food Technologists at which he read a clear and informative paper on "Food Technology in the Enforcement of the Federal Food, Drug, and Cosmetic Act."

He was a member of the Society of American Bacteriologists, Association of Official Agricultural Chemists, the Washington Academy of Science, the Institute of Food Technologists, and a Fellow of the American Public Health Association, in which organization he served as Chairman of the Food and Nutrition Section in 1940.

Dr. Hunter was known by practically all workers in every phase of the food field. He was greatly respected for his scientific ability, for his sound judgment, for his common sense, for his even temperament, and for his ability to present clearly and logically the viewpoint which he was supporting. He was a delightful speaker who knew how to win his audience. While he is gone from our midst, his life work will ever remain as a shining example of steadfast devotion to duty and responsibility, of recognition of the value of bringing the best possible equipment to our jobs, and, especially, of presenting our viewpoint in clear, unequivocal, trenchant terms.

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F. C. BLANCK.

ASCORBIC ACID, THIAMIN, RIBOFLAVIN, AND NIACIN CONTENT OF POTATOES IN LARGE-SCALE FOOD SERVICE[1]

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The purpose of this study was to determine the content of ascorbic acid, thiamin, riboflavin, and niacin in potatoes as they were peeled, soaked, steamed, mashed, and held for serving in the Navy mess hall on the Cornell University campus, where 2,800 Navy personnel were served. Samples for analyses were taken from the potatoes at various stages during the regular methods of preparation in use at the mess hall. No attempt to change these methods was made, except for the addition of salt to the soaking water of some of the potatoes.

EXPERIMENTAL PROCEDURE

The potatoes used in the first four, and in the sixth, eighth, and ninth studies were U. S. No. 1 grade, Epma brand, from Rocomoke, Md.; those in the fifth and seventh studies were also U. S. No. 1 grade but were from John Richter, Duncannon, Pa. The variety of the potatoes and the storage conditions before they were delivered were unknown. At the Navy mess hall the potatoes were stored in the shipping sacks at room temperature.

Preparation Methods: The potatoes were peeled in an institution-type potato peeler and were then held in cold water for 0 to 60 minutes before they were eyed. About 45 pounds of potatoes were drained at one time, placed on a table, and eyed by hand with a small knife. As each potato was eyed it was dropped into water [20 to 23°C.(68 to 73.4°F.)] in 31-gallon galvanized iron cans and soaked for 0 to 28.5 hours (Table 1). In Study 6 the potatoes, immediately after being eyed, were cut in large cubes (about two inches) for creaming; in each of the other studies they were left whole.

The soaked potatoes were drained, placed in perforated steamer pans 21 by 19.5 by 8.5 inches, each holding about 30 pounds, and steamed in an institution-type steamer which registered five pounds pressure.

In Studies 2, 3, and 7 the steamed potatoes were mashed. These potatoes, in about 60-pound lots (the contents of two steamer pans), were put into the bowl of a mechanical mixer as soon as they were removed from the steamer. Both the bowl and the beater were of heavy aluminum. About 1.5 pounds of fat (butter in Studies 2 and 3 and margarine in Study 7) and one-half cup of salt were added and the potatoes were mixed on low speed for about one minute. Then hot milk (exact amount could not be

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Cornell University.

obtained) was added, the mixer was turned to medium speed and run for not more than two minutes. The total time for mashing was not more than five minutes. The mashed potatoes stood at room temperature for 0 to 15 minutes until they were transferred to the steam table. Each pan held about 19 quarts of mashed potatoes. The serving temperature ranged from 65 to 72°C.(149 to 161.6°F.).

TABLE 1

Apparent Effect of Steaming, Mashing, and Holding Procedures on Percentage Retention of Vitamins in Potatoes

Study No.	Preliminary treatment of vegetable	Steaming time	Apparent retention[1]			
			Ascorbic acid	Thiamin	Riboflavin	Niacin
		min.	*pct.*	*pct.*	*pct.*	*pct.*
1	Peeled, eyed, and soaked 5 hr. 19 min..	33	120	97	73	86
		74	103	84	69	86
2	Peeled, eyed, and soaked 4 hr. 50 min..	50	139	91	100	88
	Mashed	....	64	67	129[3]	....
	Mashed, held 30 min	....	67	....	....	....
	Mashed, held 1 hr	....	60	....	....	....
3	Peeled, eyed, and soaked 5 hr. 26 min..	49	72	68	69	100
	Mashed	....	60	62	108[3]	....
	Mashed, held 30 min	....	56	....	....	....
3a[2]	Peeled, eyed, and soaked 28 hr. 30 min	65	122	79	95	96
4	Peeled, steamed 17 min. later	53	88	84	72	78
5	Peeled, eyed, and soaked 5 hr. 20 min..	45	97	128	96	86
5a[2]	Peeled, eyed, and soaked 4 hr. 5 min. in 2.5% salt solution	55	85	72	95	91
6	Peeled, eyed, sectioned, and, soaked 4 hr. 40 min	35	107	79	89	90
	Peeled, eyed, sectioned, and, soaked 4 hr. 40 min	65	97	85	94	86
7	Peeled, eyed, and soaked 19 min	38	125	88	112	92
	Mashed	....	72	68	177[3]	78
	Mashed, held 30 min	....	53	74	177[3]	78
8	Peeled, eyed, and soaked 4 hr. 20 min..	37	79	85	96	83
8a[2]	Peeled, eyed, and soaked 4 hr. 20 min. in 2.5% salt solution	37	128	76	....	95
9	Peeled, eyed	40	114	110	100	82
	Peeled, eyed, steamed, held in steamer 30 min	40	46	100	100	89
9a[2]	Peeled	43	108	89	88	92

[1] Per cent retention calculated on dry basis from vitamin content of potatoes after any soaking and immediately before steaming. [2] The potatoes used in these studies were of lots of the same number; that is, the potatoes used in 3A were of the same lot as the potatoes used in Study 3. [3] Riboflavin increases were due to that contained in the added milk, which in Study 7 contained 0.036 mg. of thiamin and 0.24 mg. of riboflavin per 100 gm. on the moist basis.

Because the length of soaking, steaming, and holding periods were subjected to variations, fortuitous or otherwise, the times for each of these procedures in each study are given (Table 1).

Sampling potatoes in a busy mess hall kitchen in which food for 2,800 men was being prepared presented difficulties not encountered in con-

trolled quantity cookery studies. Preparations had to be watched very closely in order to keep track of the one lot of potatoes selected for study and to take samples rapidly so as to avoid interference with the duties of the workers. To insure obtaining the potatoes desired, the two workers, who had had institution management training and experience, worked along with the employees.

Two to three hundred pounds of potatoes (the quantity soaked in one or two 31-gallon cans) were used as the source of all sampling for one study. Because of sampling difficulties inherent in potatoes, at least 20 potatoes of representative size were removed at each stage of sampling: unpeeled, peeled, eyed, soaked, steamed, and mashed. The whole potatoes, cubed potatoes, and scoopfuls of the mashed potatoes were removed from the top, center, and bottom of each container. The whole potatoes were rapidly quartered in bilateral sections with a sharp stainless-steel knife. One quarter-section of each potato was reserved for analyses. Bilateral halves of these quarter-sections were stored in the dark in tightly stoppered sample bottles until the sampling and observation for the day were completed. They were then taken to the laboratory where they were analyzed in duplicate for thiamin, riboflavin, niacin, and moisture. The remaining halves, weighing at least 200 grams, were analyzed for ascorbic acid in triplicate. These sections of potato were immediately and rapidly chopped on a board with a stainless-steel knife, weighed, made to volume with the extracting acid, ground in a Waring blendor, and stored in stoppered centrifuge tubes. This procedure made use of the time between sampling stages. When sampling for the day was completed, the ascorbic acid samples were returned to the laboratory for completion of the analysis. The soaking water was not analyzed and there was no cooking water.

Methods of Analysis: Ascorbic acid was determined by the method of Bessey and King (1933) and modified by the use of a Waring blendor. Freshly prepared 1N sulfuric acid containing two per cent metaphosphoric acid was used as the extractant.

Thiamin and riboflavin were determined by the combined method as outlined by Conner and Straub (1941) and modified by the thiochrome method adapted for vegetables by Moyer and Tressler (1942).

Niacin determinations were made by a microbiological method essentially that of Snell and Wright (1941). Water extraction of the niacin was used. Duplicate aliquots were set up at three-, four-, and five-ml. levels.

Total solids were determined by drying 15 to 20 grams of the potatoes at 70°C.(158°F.) under 28- to 30-inch vacuum for 48 hours.

DISCUSSION OF RESULTS

The range of vitamin content, on the moist basis, of the raw, peeled, and eyed potatoes from both sources was as follows: ascorbic acid, from 13 to 31 mg.; thiamin, from 0.087 to 0.14 mg.; riboflavin, from 0.037 to 0.083 mg.; and niacin, from 0.44 to 0.90 mg. per 100 grams. On the dry basis, the range was as follows: ascorbic acid, from 58 to 164 mg.; thiamin, from 0.38 to 0.83 mg.; riboflavin, from 0.16 to 0.36 mg.; and niacin, from 2.3 to 5.3 mg. per 100 grams. Among the possible causes of the relatively

wide ranges were, besides sampling difficulties inherent in potatoes, the different sources of the potatoes and the storage temperature and time both before and after the potatoes reached the mess hall. An inspection of the data gave no correlation between the source and the vitamin content of the potatoes used in the nine studies. The average vitamin content of the peeled and eyed potatoes on the moist basis was as follows: ascorbic acid, 19 mg.; thiamin, 0.11 mg.; riboflavin, 0.05 mg.; and niacin, 0.67 mg. per 100 grams.

Because preparation procedures were never exactly the same, each study is reported separately. No statistical treatment was possible. The apparent percentage retentions were calculated on the dry basis because it was impossible to obtain either the weight of the potatoes in any soaking or cooking lot at the various stages of sampling or the weight of the soaking water. This basis of calculation probably involved appreciable error because some of the starch, as well as some of the other water-soluble constituents, were no doubt leached into the soaking water.

During the various soaking procedures the ascorbic acid retentions ranged from 59 per cent for the potatoes soaked for 28 hours and 30 minutes to 103 per cent for those soaked for 19 minutes (Study 7). The thiamin retentions ranged from 64 per cent for the potatoes soaked for five hours and 20 minutes (Study 5) to 113 per cent for those soaked for 19 minutes. The riboflavin retentions ranged from 80 per cent for the potatoes soaked for four hours and 20 minutes (Study 8) to 118 per cent for those soaked for five hours and 26 minutes (Study 3). The niacin retentions ranged from 49 per cent for the potatoes soaked for 28 hours and 30 minutes to 122 per cent for those soaked for five hours and 20 minutes (Study 5).

As the length of soaking time increased, no consistent downward trend of percentage vitamin retentions was observed. Most of the soaking times ranged from four hours to five hours and 30 minutes. Other factors, such as the proportion of water to potatoes and the size of the potatoes, were involved. On the average, the vitamin losses during soaking were slight: 14 per cent for ascorbic acid and for niacin, eight per cent for thiamin, and six per cent for riboflavin.

The apparent percentage retentions during steaming, mashing, and holding as reported (Table 1) are calculated on the vitamin content of the potatoes after any soaking and immediately before steaming. The range of apparent retentions in the potatoes steamed for 30 to 74 minutes, after soaking in tap water for 19 minutes to five hours and 26 minutes, was as follows: ascorbic acid, from a loss of 28 to a gain of 39 per cent; thiamin, from a loss of 32 to a gain of 28 per cent; riboflavin, from a loss of 31 to a gain of 12 per cent; and niacin, from no loss to a loss of 22 per cent (Table 1).

The losses from potatoes which had not been soaked before cooking were in most cases within the above ranges. From these studies it seems that it is possible to steam potatoes, with or without soaking, and have only small losses of any of these vitamins; they ranged from no loss to maximum losses of one-fourth to one-third.

Steaming the peeled and eyed potatoes for 37 to 55 minutes after they had been soaked in 2.5 per cent salt solution for four hours and five minutes to four hours and 20 minutes resulted in apparent percentage vitamin losses which were also within the above ranges. Therefore, no conclusions can be drawn concerning the effect of soaking in salt water on vitamin retention in the steamed potatoes.

Steaming the potatoes for 30 minutes longer (second part of Study 6) resulted in no appreciable further loss of any of the vitamins. Holding steamed potatoes in the steamer with the steam turned off for 30 minutes (Study 9) apparently resulted in an appreciable loss of ascorbic acid. In the latter case, oxygen probably entered the steamer; in the former, it was kept out by the steam.

Mashing the potatoes apparently resulted in some losses of ascorbic acid; the riboflavin content was increased because of that in the added milk; the thiamin and niacin contents were little affected. Holding the mashed potatoes warm for 30 minutes resulted in further loss of ascorbic acid, probably because of the enmeshed air and the favorable temperature for oxidation of this vitamin. No change in the retention of the "B" vitamins occurred.

The most valuable data in the tables, as well as those open to the least criticism, are those giving the vitamin content of a 100-gram serving of the steamed or mashed potatoes (Table 2). The range of vitamin content of the steamed potatoes was as follows: ascorbic acid, from 11 to 31 mg.; thiamin, from 0.07 to 0.12 mg.; riboflavin, from 0.034 to 0.071 mg.; and niacin, from 0.46 to 0.76 mg. per 100-gram serving. The range of vitamin content of a 100-gram serving of the mashed potatoes was as follows: ascorbic acid, from 9.6 to 17 mg.; thiamin, from 0.084 to 0.11 mg.; riboflavin, from 0.056 to 0.10 mg. (part of this content was due to the added riboflavin-rich milk); and niacin, 0.46 mg. (one study only). Ascorbic acid was the only vitamin which varied appreciably in amount in the steamed and mashed potatoes.

SUMMARY

Raw, peeled, and eyed potatoes of two varieties contained, on the moist basis, an average of 19 mg. of ascorbic acid, 0.11 mg. of thiamin, 0.05 mg. of riboflavin, and 0.67 mg. of niacin per 100 grams.

Percentage vitamin losses in the steamed potatoes which had been soaked in tap water for 0 to 28.5 hours and steamed for 30 to 65 minutes ranged as follows: ascorbic acid, 28 to a gain of 39; thiamin, 32 to a gain of 28; riboflavin, 31 to a gain of 12; and niacin, 0 to 22. No relative trend of loss was noted either between lengths of soaking or lengths of steaming. From these studies it seems that it is possible to steam potatoes, with or without soaking, with small losses of any of these vitamins; the maximum losses ranged from about one-fourth to one-third.

Keeping either steamed or mashed potatoes warm apparently resulted in an appreciable loss of ascorbic acid; other vitamins were not appreciably affected. The vitamin content of a 100-gram serving of the steamed potatoes ranged as follows: ascorbic acid, from 11 to 31 mg.; thiamin, from 0.07 to 0.12 mg.; riboflavin, from 0.034 to 0.071 mg; and niacin, from 0.46

to 0.76 mg. The vitamin content of a 100-gram serving of the mashed potatoes ranged as follows: ascorbic acid, from 9.6 to 17 mg.; thiamin, from 0.084 to 0.11 mg.; riboflavin, from 0.056 to 0.10 mg.; and niacin, 0.46 mg. (one study only).

TABLE 2

Ascorbic Acid, Thiamin, Riboflavin, and Niacin Content of a 100-Gram Serving of Potatoes as Prepared at the Mess Hall[1]

Study No.	Description	Ascorbic acid	Thiamin	Riboflavin	Niacin
		mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*
1	Steamed 33 min. after being soaked 5 hr. 19 min.	29	.11	.038	.48
	Steamed 74 min. after being soaked 5 hr. 19 min.	26	.10	.038	.50
2	Steamed 50 min. after being soaked 4 hr. 50 min.	28	.11	.036	.46
	Mashed	17	.11	.062	....
	Mashed, held 30 min.[2]	14	....	....	....
	Mashed, held 60 min.[2]	13	....	....	....
3	Steamed 49 min. after being soaked 5 hr. 26 min.	17	.092	.036	.58
	Mashed	14	.084	.056[3]	....
3a	Mashed, held 30 min.[2]	13	....	....	....
	Steamed 65 min. after being soaked 28 hr. 30 min.	17	.092	.038	.50
4	Steamed 53 min., uneyed, within 17 min. of peeling.	17	.11	.040	.64
5	Steamed 45 min. after being soaked 5 hr. 20 min.	31	.12	.044	.48
5a	Steamed 55 min. after being soaked 4 hr. 5 min. in 2.5% salt solution	29	.12	.044	.48
6	Steamed 35 min. after being cut in cubes and soaked 4 hr. 40 min.	22	.081	.070	.57
	Steamed 65 min. after being soaked 4 hr. 40 min.[4]	19	.084	.071	.52
7	Steamed 38 min. after being soaked 19 min.	17	.12	.067	.58
	Mashed	9.6	.088	.10[3]	.46
	Mashed, held 30 min.[2]	7.0	.097	.10[3]	.46
8	Steamed 37 min. after being soaked 4 hr. 20 min.	11	.082	.048	.74
8a	Steamed 37 min. after being soaked 4 hr. 20 min. in 2.5% salt solution	12	.070	....	.76
9	Steamed 40 min., unsoaked	15	.097	.039	.53
	Held in steamer 40 min.[2]	6.2	.087	.039	.58
9a	Steamed 43 min., uneyed, unsoaked	17	.094	.034	.53

[1] All figures reported on the moist basis. [2] Holding study. [3] Increase in riboflavin content was probably due to addition of milk during mashing. [4] This might be considered a 30-minute holding period.

PRACTICAL APPLICATIONS

Apparently there was no severe loss of any of the vitamins from potatoes during soaking and steaming. Good practice, therefore, would be to prepare potatoes for cooking before preparing vegetables from which vitamins are lost rapidly when they are cut, cleaned, and held before cooking.

Keeping the mashed or steamed potatoes warm resulted in a progressive loss of ascorbic acid; thiamin, riboflavin, and niacin retentions were not appreciably affected. Long periods of holding cooked potatoes should, therefore, be avoided.

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RETENTION OF VITAMINS AND PALATABILITY IN LARGE-SCALE FOOD SERVICE OF UNSULFITED, DEHYDRATED CABBAGE[1]

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The purpose of this study was to determine the retention of ascorbic acid, thiamin, riboflavin, and niacin in cabbage during commercial dehydration of two varieties and during quantity preparation of the dehydrated cabbage (1) cooked by three methods; (2) prepared as coldslaw; (3) held after cooking; and (4) held as coldslaw at room temperature. The cabbage was also scored for palatability.

EXPERIMENTAL PROCEDURE

Both varieties of cabbage were from lots commercially dehydrated by the Dry-Pack Corporation at Lyons, New York. The cabbage was grown on upland soil within a 75-mile radius of the dehydration plant.

The first lot was of a domestic variety; the heads were firm, averaged from five to seven pounds in weight, and had a pale green color. The second lot was of the Danish Ballhead variety; the heads were more loosely packed, had coarser leaves, and a white color.

Both varieties were stored in outside bins at 17.8 to 18.9°C.(64 to 66°F.) until they were used, within 12 hours. The cabbage was trimmed, cored, and machine-cut into ⅛-inch shreds. The domestic cabbage was steam-blanched for five minutes at 82.2°C.(180°F.) and the Danish Ballhead cabbage was blanched for three minutes at 100°C.(212°F.). The cabbage was spread on stainless-steel trays and dehydrated for about five and one-half hours in a cabinet drier.

Both the domestic cabbage, which was packed in tightly covered, one-gallon tin containers, and the Danish Ballhead cabbage, which was packed in soldered, five-gallon, tin containers in an atmosphere of carbon dioxide, were stored at 0°C.(32°F.) until analyzed and cooked. All of the studies were completed within six weeks except Study 5 which was made 17 months later.

Cooking Methods: In each study except the fifth, the dehydrated cabbage was cooked in amounts to serve 50 (about two-thirds cup of cooked cabbage per person). Cooking lots of 790 grams were used. In the fifth study the cabbage was cooked in amounts to serve 50, 100, and 200 men.

Study 1—Boiled cabbage: Dehydrated cabbage of a domestic variety was cooked according to the manufacturer's directions. The cabbage was

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Cornell University.

added to 16,330 gm. of water; 45 minutes were required for the water to reach the boiling point although the kettle was placed over two institution-size gas burners. The cabbage was then simmered for 30 minutes. Proportional amounts of cabbage and cooking water were held in an institution-type electric warming oven at 60° C.(140° F.) for one and one-half hours and for three hours.

In the following studies Danish Ballhead cabbage was used.

Study 2—Coldslaw: The cabbage was refreshed in 12,700 gm. of water for 30 minutes. Then 450 gm. of a vinegar dressing, consisting of 472 gm. of sugar, 960 gm. of vinegar, 480 gm. of water, and 119 gm. of salt, was mixed with the drained cabbage. Samples were analyzed after standing at room temperature for one-half, one and one-half, and three hours.

Study 3—Boiled cabbage: The cabbage was added to 7,500 gm. of boiling water in a heavy aluminum stockpot on a gas range. The water returned to the boil in one and one-half minutes; the cabbage was stirred, covered, and allowed to simmer for 12½ minutes. It was held at about 90°C.(194°F.) in a covered, aluminum stockpot over a low gas flame for one and one-half hours and for three hours.

Study 4—Boiled cabbage: The cabbage was added to 10,000 gm. of boiling water in a steam-jacketed kettle and boiled for 10 minutes. The water did not stop boiling when the cabbage was added.

Study 5—Boiled cabbage: Cabbage was cooked in a minimum proportion of water in a steam-jacketed kettle in amounts to serve 50, 100, and 200 persons. The water did not stop boiling when the cabbage was added. The cabbage was pushed under the water with a stainless-steel skimmer and the steam was turned low so that the water just simmered for 10 minutes.

Part 1—Lots of 790 gm. were added to 7,500 gm. of boiling water in a five-gallon kettle.

Part 2—Lots of 1,580 gm. were added to 15,000 gm. of boiling water in a 15-gallon kettle.

Part 3—Lots of 3,160 gm. were added to 30,000 gm. of boiling water in a 40-gallon kettle.

Palatability Tests: Cabbage prepared by each method was scored for flavor, texture, and appearance by at least six judges who had had experience in scoring foods for palatability. Five points were allowed for each factor so that the highest score possible was 15.

Sampling: Before each cooking, analyses of the dehydrated cabbage were made. Losses during cooking were then calculated from these results in order to rule out the effect of changes owing to storage or of variation from can to can.

The uncooked, dehydrated cabbage was ground in 75-gm. lots in a Wiley mill to 20 mesh and mixed before sampling.

At each sampling stage the cabbage was drained for two minutes in an aluminum colander and the weights of the cabbage and of the liquid recorded. Spoonfuls of the drained cabbage, totaling about 400 gm., were then removed from the top, bottom, center, and sides of the colander and mixed.

Ascorbic acid determinations were made immediately. Determinations of thiamin and riboflavin were made under controlled illumination. Samples for the analysis of niacin were frozen, and analyses were made within two weeks. Aliquots of refreshing and cooking waters were also analyzed.

Methods of Analysis: Ascorbic acid was determined by the method of Bessey and King (1933), as modified by Mack and Tressler (1937), and further modified by the use of a Waring blendor.

Thiamin and riboflavin were determined by the combined method of Conner and Straub (1941) as modified for vegetables by Moyer and Tressler (1942).

Niacin determinations were made by the microbiological method as outlined by Snell and Wright (1941).

Total solids were determined by drying the cabbage at 70°C.(158°F.) under 28- to 30-inch vacuum. Two-gram samples of the uncooked, ground, dehydrated cabbage were dried for four hours; from 20 to 30 gm. of the fresh and cooked cabbage, for 36 hours.

The per cent retentions were calculated on the basis of the vitamin content of the total lot of vegetable and cooking water at each stage of sampling.

Statistical Treatment: A statistical analysis of representative data was made to determine whether the observed differences between the effects of the different cooking and holding methods on the vitamin contents were real or were due merely to experimental variation in the methods of study.

The per cent retention of vitamin in the cabbage as served, as the figure of greatest importance in the studies, was selected for the statistical treatment as outlined by Snedecor (1938). Standard errors were calculated and used in ascertaining by the "t-value" method the significance, at the five-per cent level, of the difference of the observed retention of each vitamin between (a) the different cooking methods, (b) the different holding methods, if any, and (c) the cooking methods and the holding methods.

DISCUSSION OF RESULTS

The dehydrated cabbage of a domestic variety contained an average of 250 mg. of ascorbic acid, 0.59 mg. of thiamin, 0.47 mg. of riboflavin, and 3.0 mg. of niacin per 100 gm., on the dry-weight basis. The Danish Ballhead cabbage contained an average of 187 mg. of ascorbic acid, 0.57 mg. of thiamin, 0.38 mg. of riboflavin, and 2.8 mg. of niacin per 100 gm. The content ranged from 164 to 201 mg. for ascorbic acid; from 0.49 to 0.65 mg. for thiamin; from 0.36 to 0.40 mg. for riboflavin; and from 2.8 to 3.0 mg. for niacin.

After 17 months' storage in carbon dioxide at 32°F., the content of ascorbic acid at 204 mg., of thiamin at 0.63 mg., and of riboflavin at 0.40 mg. per 100 gm. was within the range of the contents in the dehydrated cabbage before storage. The niacin was apparently higher at 3.8 mg. per 100 gm.

Almost one-half of the ascorbic acid was lost during the dehydration of the domestic cabbage, and about one-third during the dehydration of the Danish Ballhead cabbage (Table 1). The losses of thiamin, riboflavin, and niacin, however, were relatively small for each variety. These small

TABLE 1

Effect on Vitamin Retention of Dehydration of Cabbage and of Preparation and Holding Procedures

Condition of vegetable	Average vitamin retention[1] ± S.E.[2]								Number of replications
	Ascorbic acid		Thiamin		Riboflavin		Niacin		
	Cabbage	Liquid	Cabbage	Liquid	Cabbage	Liquid	Cabbage	Liquid	
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	
Dehydrated (domestic)	53[3]	....	95[3]	....	103[3]	....	86[3]	....	1
Dehydrated (Danish Ballhead)	70[3]	....	94[3]	....	86[3]	....	52[3]	....	1
1. a. Boiled (domestic)	6±1.3	20	42±1.0	46	61±1.5	56	23±4.2	61	5
b. Held 90 min.	5±1.5	6	50±1.4	39	81±3.8	48	28±4.9	58	5
c. Held 180 min.	4±0.5	4	49±2.7	36	74±8.8	45	35±4.0	50	4
2. a. Soaked (Danish Ballhead)	37	52	51	43	63	39	35	62	4
b. Coldslaw	26±2.3	4	52±5.4	6	58±4.7	6	24±1.8	8	4
c. Held 90 min.	24±2.3	3	45±2.1	4	62±2.2	6	20±2.1	7	4
d. Held 180 min.	21±2.3	2	48±2.9	3	67±1.4	4	24±2.8	4	4
3. a. Boiled (Danish Ballhead)	17±1.4	28	76±6.1	15	76±3.9	27	68±3.7	48	5
b. Held 90 min.	12±0.8	15	81±1.8	12	116±7.0	20	83±1.2	32	4
c. Held 180 min.	9±0.11	6	82±3.6	8	100±5.8	19	82±3.7	33	4
4. a. Boiled (Danish Ballhead)	14±1.2	40	58±4.7	45	74±2.5	43	41±6.7	51	5
5. a. Boiled[4] (Danish Ballhead)	22±1.8[5]	27	66±3.8[5]	31	79±1.7[5]	38	70±2.1[5]	36	5
	26[6]	24[6]	70[6]	27[6]	83[6]	32[6]	73[6]	33[6]	1
	20[7]	28[7]	71[7]	33[7]	76[7]	36[7]	72[7]	39[7]	2
	21[8]	27[8]	58[8]	32[8]	81[8]	48[8]	66[8]	35[8]	2

[1] Per cent retention was calculated on the moist basis using weights of the uncooked and the cooked vegetable and the cooking water. [2] Significant differences in retention were found between:

Ascorbic acid

5a and 1a, 4a, 1b, 1c, 3b, 3c
1a and 2b, 3a, 4a, 2c, 3b, 2d, 3c
2b and 4a, 3a, 1b, 3b, 1c, 3c
1b and 2c, 3b, 3a, 4a, 3c
2c and 3b, 4a
1c and 2d, 3c, 3a, 4a, 2c, 3b
2d and 3c, 1b, 3b
3a and 3b, 3c
3c and 4a, 2c, 3b

Riboflavin

1a and 4a, 3a, 1b, 3b, 3c, 2d
3b and 2b, 3a, 4a, 1c, 2d, 2c
3c and 2b, 3a, 4a
1b and 2d, 3c
5a and 1a, 2a, 2c, 3b, 3c

Thiamin

3a and 1a, 4a, 2c, 2d
1b and 3b, 2c, 1a, 3c
2c and 3b
3c and 1c, 2d, 1a, 2b, 4a, 2c
3b and 1a, 2b, 4a, 2d, 1c
5a and 1a, 1b, 1c, 2b, 2c, 3b

Niacin

3a and 1a, 2b, 4a, 1b, 2c, 3b, 1c, 2d
3b and 1a, 2b, 1c, 2d, 1b, 2c
3c and 1a, 2b, 4a, 1b, 1c, 2d
2c and 1c, 3c
5a and 1a, 1b, 1c, 2a, 2b, 2c, 3b, 3c, 4a

[3] On the dry basis. [4] The cabbage used in this study was of the same lot as that used in former studies but had been stored in the original unopened tins for 17 months at 0°C.(32°F.). [5] The figures given are the averages of the percentages found when the cabbage was cooked in amounts to serve 50, 100, and 200 by the same method. [6] Retention in amounts to serve 50. [7] Retention in amounts to serve 100. [8] Retention in amounts to serve 200.

losses probably resulted at least partially because the cabbage was steam-blanched rather than water-blanched.

The range of percentage retention of the vitamins during the several preparation methods was as follows: ascorbic acid, from 6 to 22 per cent; thiamin, from 42 to 76 per cent; riboflavin, from 58 to 79 per cent; and niacin, from 23 to 70 per cent.

Cooking the cabbage by adding it to the smallest amount of boiling water and boiling it for a relatively short time (Studies 3 and 5) resulted in a greater percentage retention of each of the four vitamins than did cooking the cabbage by the other methods. The vitamin retentions were very similar in Studies 3 and 5 in which the proportion of vegetable to water was the same although the former was cooked in a stockpot on a gas range for 14 minutes and the latter in a steam-jacketed kettle for 10 minutes. The palatability rating of the cabbage cooked by Method 3 was higher than that for the cabbage cooked for the longest time in the largest amount of water (Study 1), but lower than for the cabbage cooked in the steam-jacketed kettle for the shortest time in an intermediate amount of water (Study 4, Table 2).

The cabbage cooked by Method 5 scored lower than did the cabbage cooked by Methods 3 and 4. This lower score was probably due to changes that occurred during the 17 months' storage. The cabbage was considered acceptable. Although it had changed little in flavor and appearance, it had become more chewy. Increasing the amount of cabbage cooked at one time from enough to serve 50 to enough to serve 100 and 200, in proportional amounts of water, had little observable effect on the percentage retention of the vitamins or on the palatability (Tables 1 and 2). The range of vitamin retention in the cabbage cooked in the three amounts was within the ranges of retention of that cooked by other methods.

Considerable ascorbic acid was destroyed during cooking by every method, ranging from 46 per cent in the cabbage boiled for 10 minutes (Study 4) to 75 per cent in the cabbage cooked for 75 minutes (Study 1).

Almost complete destruction of both the retained and dissolved vitamin occurred when the cabbage was cooked for 75 minutes (Study 1). Actual destruction of thiamin, riboflavin, and niacin did not occur to an appreciable extent in any of the cooking methods. Increasing the amount of cooking water, however, significantly decreased the vitamin content of the drained, cooked vegetable and increased that found in solution.

The coldslaw retained a higher percentage of ascorbic acid, even after being held at room temperature for three hours, than did any of the cooked cabbage. The percentage retentions of thiamin, riboflavin, and niacin were slightly higher than in Method 1, but lower than in Methods 3, 4, and 5, owing to solution losses during refreshing. The coldslaw was a very acceptable product, rating considerably higher in color and flavor than any of the cooked cabbage.

During holding at 90°C. the retention of ascorbic acid in the cabbage cooked by Method 3 decreased. The loss of this vitamin during cooking by Method 1 was already so great that holding at 60°C. did not produce an appreciable further decrease. No other significant changes in any of the vitamins were observed in holding studies. A marked loss of palatability,

TABLE 2

Vitamin Content and Palatability Score of a Serving (100 Grams) of Dehydrated Cabbage

Method of preparation	Treatment	Time	Proportion of vegetable to liquid by weight	Vitamin content				Palatability score[1]
				Ascorbic acid	Thiamin	Riboflavin	Niacin	
		min.		*mg.*	*mg.*	*mg.*	*mg.*	
Boiled on top of stove	1. Added to cold water	....	1:20.5					
	Brought to boil	45						
	Simmered[2]	30	1: 1.5	1.6	.037	.033	.090	9
	Held at 60°C.(140°F.)	90	1: 1.1	1.1	.040	.038	.10	....
	Held at 60°C.(140°F.)	180	1: 0.96	0.92	.039	.034	.11	....
	3. Added to boiling water	....	1: 9.5					
	Returned to boil	1.5						
	Simmered[3]	12.5	1: 0.58	4.7	.066	.044	.28	10
	Held at 90°C.(194°F.)	90	1: 0.31	2.8	.067	.060	.30	....
	Held at 90°C.(194°F.)	180	1: 0.31	2.4	.064	.056	.31	....
Boiled in steam-jacketed kettle	4. Added to boiling water	....	1:16.1					
	Simmered[3]	10	1: 0.98	3.5	.043	.040	.19	11
	5. Added to boiling water	....	1: 9.5					
	Simmered[4]	10	1: 0.57	6.9	.064	.049	.41	9
Coldslaw	2. Added to cold water	....	1:16.1					
	Rehydrated at 22.2°C.(72°F.)	30	1: 2.1					
	Marinated[3]	30	1: 0.21	8.3	.048	.030	.10	12
	Held at 22.2°C.(72°F.)	90	1: 0.14	6.7	.034	.034	.083	....
	Held at 22.2°C.(72°F.)	180	1: 0.09	5.8	.035	.036	.096	....

[1] The possible score was 15, five each for appearance, texture, and flavor. [2] A domestic variety. [3] Danish Ballhead variety. [4] From the same lot of Danish Ballhead cabbage used on previous studies, but stored for 17 months at 0°C.(32°F.).

however, resulted; the cabbage turned a red-brown color, was undesirable in flavor and aroma, and became water-soaked. The coldslaw, on the other hand, did not deteriorate in palatability upon standing nor was the vitamin content appreciably affected.

Increases in riboflavin and niacin during holding may be attributable to sampling error, incomplete extraction from the uncooked, dehydrated vegetable, and breakdown of compounds during holding into forms that interfere with the methods of analysis. The method of analysis for riboflavin, Conner and Straub (1941), was checked by the Snell and Strong (1939) microbiological method at both the New York State Experiment Station at Geneva, New York, and at the Federal Nutrition Laboratory at Ithaca, New York. The results of this comparison were in fairly good agreement.

The average palatability score and vitamin content of a 100-gram serving of dehydrated cabbage after the five methods of preparation are given (Table 2).

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RETENTION OF VITAMINS AND PALATABILITY IN LARGE-SCALE FOOD SERVICE OF SULFITED, DEHYDRATED CABBAGE[1]

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The purpose of this study was to determine the effect on palatability and on the retention of ascorbic acid, thiamin, riboflavin, and niacin of preparing sulfited, dehydrated cabbage in amounts to serve 50 by five methods of boiling, by two methods of preparation as coldslaw, of holding the boiled cabbage and coldslaw for one and one-half hours, and of holding some of the dehydrated cabbage in unopened containers for three and four months at room temperature and then boiling it by one method. The object was to determine the palatability and the vitamin content of the cabbage as served and also the factors in preparation that affect the vitamin retention. A further purpose was to compare the vitamin content and the palatability of prepared, sulfited cabbage with that of unsulfited cabbage—Fenton, Gleim, Albury, McCartney, and Visnyei (1946).

EXPERIMENTAL PROCEDURE

The sulfited cabbage used in the first six studies was of the Penn State, Glory of Enkhiuzen, or Flat Dutch variety. It was steam-blanched for two and two-thirds minutes at 99 to 100°C.(210.2 to 212°F.) on wooden trays. It was sprayed at the end of the first one-third of the blanching with a mixture of equal amounts of sodium sulfite and sodium metabisulfite, so that the final SO_2 concentration of the spray was approximately 0.18 per cent. The cabbage was dehydrated in a single-stage, counter-current tunnel for about 11 hours with a finishing temperature of 60°C.(140°F.). It was then detrayed, slightly warmed, and packed in five-gallon tins, seven pounds net weight, in carbon dioxide by the vacuum method. The cabbage was dehydrated by the Puccinelli Packing Company, Turlock, California, on February 12, and on April 4 it was received in the laboratory where it was placed in storage at 0°C.(32°F.) until used.

The sulfited cabbage used in the last three cooking studies had been packed in five-gallon tin containers, each containing 15 pounds, by the Huston-Baetz Company at Santa Ana, California, in September, 1944. It was more finely divided than the previous sulfited cabbage, more tightly packed, and less bulky.

Cooking Methods: The cabbage was prepared in amounts to serve 50, and each method was repeated five times. The same amount of cabbage, 790 gm., was used in each study except Method 3 in which 567 gm. were used.

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Cornell University.

Method 1: Boiled cabbage was prepared according to the directions found in the can which were the same as those found in the War Department Technical Manual TM10-406, "Cooking Dehydrated Foods." The bacon, salt, and pepper were omitted. The cabbage was added to 7,260 gm. of water at 20°C.(68°F.) in a 25-quart, covered, aluminum stockpot and soaked 20 minutes. The rehydrated cabbage and water were slowly brought to the boil over a gas flame (40 minutes) and the cabbage was simmered for 15 minutes. During cooking, the cabbage was stirred twice with a stainless-steel skimmer. Proportional amounts of cabbage and cooking water were placed in an aluminum stockpot and kept warm in an institution-type warming oven at about 60°C. for one and one-half hours.

Method 2: Boiled cabbage was prepared by adding the cabbage to 11,390 gm. of boiling water in a five-gallon, steam-jacketed kettle. The boiling did not stop when the cabbage was added; the cabbage was boiled for seven minutes. Proportional amounts of cabbage and cooking water were returned to the steam-jacketed kettle and held at a simmer for one hour.

Method 3: Coldslaw was prepared by rehydrating the cabbage in 5,440 gm. of water in the refrigerator [0 to 1.8°C.(32 to 35°F.)] for four hours *(Army Cooking Manual).* One-third of the drained, rehydrated cabbage was marinated in 720 gm. of a dressing made according to the directions in the *Army Cooking Manual* except that the onions were omitted. Sufficient dressing was made at one time for the complete study. It contained 9,040 gm. of vinegar, 852 gm. of salt, and 7,280 gm. of water. Another one-third portion of the drained, rehydrated cabbage was marinated for 30 minutes in the refrigerator in 450 gm. of a dressing containing 960 gm. of vinegar, 480 gm. of water, 472 gm. of sugar, and 119 gm. of salt. This was the same kind of dressing that had been used to prepare coldslaw from dehydrated, unsulfited cabbage—Fenton *et al.* (1946).

Method 4: Coldslaw was prepared following the same procedure and proportions used in preparing coldslaw from unsulfited cabbage—Fenton *et al.* (1946). The cabbage was rehydrated in 12,700 gm. of water at room temperature [29.4 to 33.3°C.(85 to 92°F.)] for 30 minutes. Four equal portions, one-fifth of the total weight of the drained, rehydrated cabbage, were marinated in 360 gm. of dressing for one-half hour and for two hours at room temperature, and for one-half hour and for two hours in the refrigerator at 0 to 1.8°C.

Method 5: Boiled cabbage was prepared by adding the cabbage to 7,500 gm. of boiling water in a 25-quart, aluminum stockpot. The water did not stop boiling; the cabbage was simmered for seven minutes.

Method 6: Boiled cabbage was prepared by adding the cabbage to the cooking water (about 2,200 gm.) drained from the cabbage cooked by Method 5 plus added tap water to make 7,500 gm. The water did not stop boiling; the cabbage was simmered for seven minutes.

The cabbage used for the following studies was from the Huston-Baetz Co.

Method 7: Boiled cabbage was prepared by adding the cabbage to 7,500 gm. of boiling water in a five-gallon, steam-jacketed kettle. The water did not stop boiling; the cabbage was simmered for seven minutes.

Method 8: Boiled cabbage was prepared from the same cabbage and by the same method used in Method 7 except that the cabbage had been stored at room temperature for three months in an unopened, five-gallon tin.

Method 9: Boiled cabbage was prepared as in Method 8 except that the cabbage had been held in an unopened container at room temperature for four months.

Sampling: Before each cooking, analyses of the dehydrated cabbage were made. Losses during cooking were then calculated from these results in order to rule out the effect of changes owing to storage or of variation from can to can.

The uncooked, dehydrated cabbage was ground in 75-gm. lots in a Wiley mill to 20 mesh and mixed before sampling.

When the cabbage cooked in the steam-jacketed kettle was done, the steam was turned off, the drain was opened, and the cabbage was transferred to an aluminum colander with a stainless-steel skimmer and allowed to drain. Five minutes were allowed for this complete procedure. At each sampling stage of the cabbage prepared by other methods it was dumped into an aluminum colander and allowed to drain for two minutes. Spoonfuls of the drained cabbage, totaling about 400 gm., were removed from the top, bottom, center, and sides of the colander and mixed.

Portions of this composite sample were immediately weighed and immersed in chilled extractive for determination of ascorbic acid. Samples for thiamin and riboflavin determinations were weighed in a room with controlled illumination. Samples for the determination of niacin were taken immediately and either analyzed at once or stored in the form of the sterile extractive until analyses were made (within three weeks). Aliquots of refreshing and cooking waters were also analyzed.

Palatability Tests: The prepared cabbage was scored for flavor, texture, and appearance by at least six experienced judges. Five points were allowed for each factor so that the highest score possible was 15.

Methods of Analysis: Ascorbic acid was determined by the method of Bessey and King (1933) as modified by Mack and Tressler (1937) and further modified by the use of a Waring blendor. Three per cent metaphosphoric acid was used as the extractant, and acetone was added to the aliquot of the extract just before titration in order to prevent the action of SO_2 with the dye—Mapson (1941).

Thiamin and riboflavin were determined by the combined method of Conner and Straub (1941) as modified for vegetables by Moyer and Tressler (1942).

Niacin determinations were made by the microbiological method of Snell and Wright (1941).

Total solids determinations were made by drying the cabbage at 70°C. (158°F.) under 28- to 30-inch vacuum. Two gram samples of the ground dehydrated cabbage were dried for six hours; from 20 to 30 gm. of the prepared cabbage and about 20 gm. of the liquid, which had been evaporated to a sirupy consistency on a low-temperature hot plate, were dried for 48 hours.

Statistical Treatment: A statistical analysis was made of the per cent retention of each vitamin in the cabbage as served, to determine whether the observed differences were real or merely due to experimental variation. Standard errors were calculated and used in ascertaining, by the "t-value" method as outlined by Snedecor (1938), the significance, at the five-per cent level, of the difference between (a) the different cooking methods, (b) the different holding methods, and (c) the cooking and the holding methods.

DISCUSSION OF RESULTS

Vitamin Content: The cabbage packed by the Puccinelli Packing Company contained an average of 376 mg. of ascorbic acid, ranging from 360 to 392; 0.15 mg. of thiamin, ranging from 0.13 to 0.16; 0.38 mg. of riboflavin, ranging from 0.33 to 0.45; and 3.0 mg. of niacin, ranging from 2.8 to 3.3 mg. per 100 gm. on the dry basis.

The ascorbic acid content ranged from 86 mg. in the coldslaw prepared by Method 4c to 256 mg. per 100 gm., dry basis, in the cabbage boiled on top of the stove in a minimum amount of liquid consisting partly of drained cooking water (Method 6).

After preparation and holding the cabbage contained so little thiamin (0.042 to 0.11 mg.) and riboflavin (0.16 to 0.38 mg.) per 100 gm., on the dry basis, that the results of chemical analysis have little practical value. The determinations were made, however, to note the effect of the sulfite on the vitamin content of the cabbage during preparation and as a basis for comparison with the content of unsulfited cabbage which had been studied in this laboratory previously—Fenton *et al.* (1946).

The niacin content ranged from 1.0 mg., in the coldslaw prepared by Method 4c, to 2.8 mg. in the cabbage boiled by Method 6.

Vitamin Retention: The effect of six methods of preparation and four methods of holding on the percentage vitamin retention are given (Table 1).

During rehydration as much as half of each of the water-soluble vitamins was, in some cases, leached out into the soaking water. During rehydration for 20 minutes in the smallest proportion of water (Method 1a) and during rehydration at 32 to 35°F. for four hours in a similarly small proportion of water (Method 3a) the cabbage retained a significantly higher percentage of ascorbic acid and of niacin than did the cabbage rehydrated in the larger amount of water (Method 4a) at 85 to 92°F. for 30 minutes. Less loss to solution occurred. The temperature and time of holding seemed of little importance compared with the proportion of water. No significant difference in the thiamin retention occurred.

During boiling and marinating the cabbage boiled by Method 5 and the coldslaw prepared by Methods 3b and 3c, in which small proportions of water were used, retained significantly more ascorbic acid than the cabbage boiled by Method 2 and the coldslaw prepared by Methods 4b, 4c, 4d, and 4e. The cabbage boiled in a small proportion of liquid which was partly drained cooking water (Method 6) retained 61 per cent ascorbic acid, which was slightly more than the 54 per cent retained in the cabbage boiled in the same amount of water (Method 5) and more than in the cabbage prepared by any other method. The boiled cabbage prepared by

TABLE 1

Effect of Preparation and of Holding on Vitamin Retention in Dehydrated, Sulfited Cabbage[1]

TABLE 1

Effect of Preparation and of Holding on Vitamin Retention in Dehydrated, Sulfited Cabbage [1]

Method	Preparation of cabbage	Average vitamin retention [2]							
		Ascorbic acid		Thiamin		Riboflavin		Niacin	
		Vegetable	Liquid	Vegetable	Liquid	Vegetable	Liquid	Vegetable	Liquid
		pct.±*S.E.*[3]	*pct.*	*pct.*±*S.E.*[3]	*pct.*	*pct.*±*S.E.*[3]	*pct.*	*pct.*±*S.E.*[3]	*pct.*
1. Boiled on top of stove	a. Rehydrated in water at 20°C.(68°F.) for 20 min	60±2.4	39	67±3.5	35	68±4.7	39	66±3.0	40
	b. Brought to boil in covered stockpot (40 min.) and simmered 15 min	48±3.1	25	33±3.8	13	83±6.7	29	76±1.6	26
	c. Held in institution-type warmer at 60°C.(140°F.) for 90 min	47±1.7	14	31±5.1	8	79±3.0	22	80±1.6	16
2. Boiled in steam-jacketed kettle	a. Added to boiling water and boiled 7 min	36±1.8	47	36±1.4	28	51±3.3	53	52±2.5	52
	b. Held at simmer	35±3.1	21	29±1.3	10	57±4.5	33	65±3.1	27
3. Coldslaw	a. Rehydrated at 0-1.7°C.(32-35°F.) for 4 hr	56±.82	33	70±3.1	34	.....	....	74±2.1	41
	b. Made into coldslaw by method in *Army Cook Book* and marinated at 32-35°F. for 30 min	41±.31	46	56±5.3	50	.....	....	60±1.8	59
	c. Made into coldslaw using dressing containing sugar and marinated at 32-35°F. for 30 min	46±.11	43	61±2.5	45	.....	....	63±1.4	54
4. Coldslaw	a. Rehydrated at 29.4-33.3°C. (85-92°F.) for 30 min	39±1.4	55	57±3.6	55	.....	....	51±1.8	62
	b. Made into coldslaw using same dressing as 3c and marinated at 85-92°F. for 30 min	31±1.5	60	46±5.2	63	.....	....	45±.43	68
	c. Same as 4b except marinated for 2 hr	30±1.5	59	52±2.8	61	.....	....	43±1.5	67
	d. Same as 4b except marinated at 32-35°F. for 30 min	31±1.4	60	58±4.3	62	.....	....	46±1.2	68
	e. Same as 4d except marinated 2 hr	33±1.8	58	55±3.5	61	.....	....	46±1.8	66
5. Boiled on top of stove	a. Added to boiling water and simmered 7 min	54±1.8	33	51±2.4	29	55±4.4	31	65±5.6	43
6. Boiled on top of stove	a. Added to combined (boiling) cooking liquid and water and simmered 7 min	61±1.5	36	58±6.4	30	58±4.6	40	64±2.8	50

[1] Five replications were made except for Method 1c and thiamin and riboflavin determinations in Method 1a, which were repeated four times, and niacin determinations in Methods 5 and 6, which were repeated three times. [2] The per cent retention and solution were calculated on the total weights of the dehydrated, rehydrated, cooked, and held cabbage, and cooking water and dressing in each lot. [3] Significant differences in retention were found between:

Ascorbic acid
4a and 1a, 3a
1b and 2a, 4b, 4c, 4d, 4e
1c and 2b, 4b, 4c, 4d, 4e
3b and 2a, 3c, 4b, 4c, 4d, 4e
3c and 2a, 2b, 4b, 4c, 4d, 4e
5 and 2a, 2b, 3b, 3c, 4b, 4c, 4d, 4e
6 and 1b, 1c, 2a, 2b, 3b, 3c, 4b, 4c, 4d, 4e

Riboflavin
1b and 2a, 2b, 5, 6
1c and 2e, 2b, 5

Thiamin
1b and 3b, 3c, 4c, 4d, 4e
1c and 3b, 3c, 4c, 4d, 4e
2a and 3b, 4c, 4d, 4e
2b and 2a, 3b, 3c, 4b, 4c, 4d, 4e
3c and 2a, 4b
5 and 1b, 1c, 2a, 2b, 3c
6 and 1b, 1c, 2a, 2b

Niacin
4a and 1a, 3a
1b and 4b, 4c, 4e
1c and 4b, 4c, 4d, 4e, 6
2a and 1b, 1c, 2b, 3c, 4c
2b and 1b, 1c, 4b, 4c, 4d, 4e
3b and 1b, 1c, 4b, 4c, 4d, 4e
6 and 4c, 4d, 4e

Method 1 retained a significantly higher percentage of ascorbic acid, 48; riboflavin, 83; and niacin, 76, than did the cabbage boiled in a larger proportion of water (Method 2) and a significantly higher percentage of ascorbic acid than the coldslaw rehydrated in the larger proportion of water (Method 4). Coldslaw prepared by Method 3b retained significantly more niacin than that prepared by Methods 4b, 4c, 4d, or 4e.

Slightly greater ascorbic acid retention occurred in the coldslaw prepared by Method 3c than in that prepared by Method 3b which used a greater amount of dressing. The greater retention was, no doubt, due to less solution. The difference in thiamin and niacin retentions was not significant. The temperature of marination made no significant difference in the ascorbic acid, thiamin, or niacin retentions.

The thiamin retention, 51 and 58 per cent, in the cabbage boiled in the smaller proportion of liquid for only seven minutes (Methods 5 and 6) and in any of the coldslaw ranging from 46 to 61 per cent retentions was significantly higher than the 33 per cent in the cabbage boiled for the longer time (Method 1) and the 36 per cent in the cabbage boiled in the larger amount of water for the same time (Method 2). Apparently heat increased the destructive effect of the sulfiting on the thiamin, and a larger proportion of water resulted in an increased loss to solution.

Because the fluorometer had to be sent for repairs, a comparison of riboflavin retention was possible in only four methods of cooking—1, 2, 5, and 6. In these, the range of retention was 51 to 83 per cent. Apparently the only loss which occurred was to solution. The highest retention resulted in the method in which the smallest proportion of water was used (Method 1) and the lowest where the opposite was true (Method 2).

During holding in its own cooking water, the cabbage boiled by either Method 1 or 2, no significant change in ascorbic acid retention occurred. The percentage of ascorbic acid found in the cooking water, however, decreased by about half. The total loss of ascorbic acid increased from 27 to 39 per cent in Method 1, and from 17 to 44 per cent in Method 2. Possibly the evaporation of the cooking water during holding kept the concentration of ascorbic acid in the water at about the same level as that in the vegetable cells so no further solution loss from the vegetable occurred. The total destruction of ascorbic acid during the preparation of coldslaw by either method was only about 10 per cent. The stability of ascorbic acid in both the coldslaw and the cooked and held cabbage is probably due to the sulfiting treatment.

Simmering the boiled cabbage for one hour resulted in a decrease in the retention of thiamin in both vegetable and liquid. No apparent destruction of thiamin occurred, however, during any of the methods of holding or of preparation of coldslaw. Evidently the sulfiting of cabbage did not affect the stability of thiamin unless heat was present.

Riboflavin retention in the boiled cabbage was not changed significantly during holding. No appreciable destruction of riboflavin occurred during either boiling or holding.

During holding of the boiled cabbage in its cooking water for one and one-half hours at 140°F. in the enclosed warmer, and during marinating the coldslaw prepared by Method 4 for two hours at either 85 to 92°F. or

32 to 35° F., no significant change in niacin retention occurred. During holding of the cabbage at a simmer for one hour, however, the percentage retention in the boiled cabbage increased significantly and that in the cooking water decreased. This may have been due to evaporation of the cooking water. The total loss of niacin was 18 per cent. No appreciable destruction of niacin occurred during any other method of preparation or holding.

Stored Cabbage: The vitamin content and the effect of cooking on retention of vitamins in the sulfited cabbage that had been stored in unopened tins at room temperature for three and four months are given (Table 2). This sulfited cabbage, which was received from the Huston-Baetz Company, contained at the beginning of storage 373 mg. of ascorbic acid, 0.17 mg. of thiamin, 0.39 mg. of riboflavin, and 3.2 mg. of niacin per 100 gm. on the dry basis. These amounts were similar to those found in the sulfited cabbage used in the other part of this study. During storage for three and for four months this cabbage apparently lost an appreciable amount of ascorbic acid and some niacin. Thiamin and riboflavin data were not obtained.

During cooking, this unstored cabbage apparently retained a higher percentage of ascorbic acid, 72, and a somewhat higher per cent of thiamin, 58, riboflavin, 85, and niacin, 74, than did the sulfited cabbage from the Puccinelli Packing Co. cooked by Method 5, which retained ascorbic acid 54, thiamin 51, riboflavin 55, and niacin 65 per cent. The amount of cooking water and the cooking times were the same, but the Huston-Baetz cabbage was cooked in a steam-jacketed kettle from which there was more evaporation and, therefore, less loss to solution. Thiamin was the only vitamin which suffered much actual destruction in either study. After storage at room temperature for three and four months the percentage loss of ascorbic acid during cooking increased.

Palatability and vitamin content of 100-gram servings of the sulfited cabbage from both sources are given (Table 3).

Boiled Cabbage: A 100-gram serving of the sulfited cabbage prepared by Method 6 (boiling in drained cooking water and tap water) contained 35 mg. ascorbic acid and 0.39 mg. of niacin, which was more than the cabbage prepared by any other method. Because of a slightly strong flavor its palatability score was slightly lower than that for the cabbage cooked by a similar method which used only tap water, Method 5. The cabbage boiled by Method 1 in a minimum amount of water for a longer time contained 25 mg. of ascorbic acid and 0.33 mg. of niacin. Its palatability score was the lowest of all the boiled cabbage, however, because of a waterlogged texture and a sulfite flavor. The cabbage boiled in the largest proportion of water scored the highest in palatability because of its mild sulfite flavor but contained less ascorbic acid and niacin.

The boiled cabbage of a different lot, Method 7, had about the same ascorbic acid and niacin content and the same intermediate score as the cabbage prepared by Method 6. This cabbage, however, when cooked after storage at room temperature, Methods 8 and 9, contained much less ascorbic acid. Its palatability decreased very slightly. Apparently the sulfite flavor was not lost appreciably during storage. All of this lot of cabbage

TABLE 2

Effect of Storing Unopened Tins of Sulfited Cabbage for Three and Four Months at Room Temperature on Retention of Vitamins During Cooking

Condition of vegetable	Storage time	Cooking method No.	Total solids	Vitamin content[1]		Vitamin retention			
				Ascorbic acid	Niacin	Ascorbic acid		Niacin	
						Vegetable	Liquid	Vegetable	Liquid
	mo.		*pct.*	*mg./100 gm.*	*mg./100 gm.*	*pct.±S.E.*	*pct.*	*pct.±S.E.*	*pct.*
	0	....	98	373	3.2	.....	....	....	....
Dehydrated	3	....	98	291	2.7	78[1]	....	85[1]	....
	4	....	97	271	1.7	73[1]	....	54[1]	....
Boiled[2]; 790 gm. added to 7,500 gm. boiling water in steam-jacketed kettle. Simmered 7 min.	0	7	12	307	2.7	72±1.4[3, 4]	23[3]	74±2.3[3, 4]	26[3]
	3	8	12	214	1.8	60±1.3[3, 4]	26[3]	54±2.4[3, 4]	22[3]
	4	9	11	190	1.6	57±.91[3, 4]	25[3]	74±2.9[3, 4]	29[3]

[1] Dry basis. [2] Each cooking method was repeated five times. [3] Calculated on weights of cabbage before and after cooking and the weight of the cooking water.
[4] Significant differences were found between:

Ascorbic acid	Niacin
7 and 8, 9	7 and 8
	8 and 9

TABLE 3

Vitamin Content and Palatability Score of a Serving (100 Grams) of Sulfited, Dehydrated Cabbage After Preparation

Method of preparation	Treatment	Time	Vitamin content per 100 gm.				Palatability score [1]
			Ascorbic acid	Thiamin	Riboflavin	Niacin	
		min.	*mg.*	*mg.*	*mg.*	*mg.*	
Boiled on top of stove	1.[2] Rehydrated at 20°C.(68°F.)	20	....	.....	.....	....	.....
	Brought to boil	40	....	.....	.....	....	.....
	Simmered	15	25	.0063	.047	.33	8.7
	Held at 60°C.(140°F.)	90	22	.0052	.046	.32	.....
	5.[2] Added to boiling water and simmered	7	30	.011	.032	.28	11.4
	6.[2] Added to boiling cooking water and liquid and simmered	7	35	.013	.036	.39	10.7
Boiled in steam-jacketed kettle	2.[2] Added to boiling water and boiled	7	20	.0079	.024	.22	11.0
	Held at simmer	60	19	.0062	.026	.27	5.6
	7.[3] Added to boiling water and simmered	7	38	.013	.045	.32	10.8
	8.[3] Added to boiling water and simmered	7	25	.....	.....	.21	10.0
	9.[3] Added to boiling water and simmered	7	22	.....	.....	.18	9.2
Coldslaw	3.[2] Rehydrated at 0-17°C.(32-35°F.)	240	....	.....	.....	....	.....
	Marinated at 32-35°F. in "Army" dressing	30	18	.011	.026	.20	10.4
	Marinated at 32-35°F. in dressing with sugar	30	24	.013	.026	.22	9.9
	4.[2] Rehydrated at 29.4-33.3°C.(85-92°F.)	30	....	.....	.....	....	.....
	Marinated at 85-92°F	30	16	.0083	.....	.18	11.7
	Marinated at 32-35°F	30	16	.010	.....	.18	11.6
	Held at 85-92°F	90	15	.0091	.....	.17	12.8
	Held at 32-35°F	90	16	.0098	.....	.18	13.0

[1] The possible score was 15, five each for appearance, texture, and flavor. [2] Cabbage received from Puccinelli Packing Co. [3] Cabbage received from Huston and Baetz Co. In Studies 8 and 9 this cabbage was stored in unopened tins at room temperature for three and four months, respectively.

might have been scored higher if it had not been so finely divided owing to tight packing in the containers.

Although the boiled cabbage which was held by either Method 1 or 2 showed no appreciable change in ascorbic acid, niacin, or riboflavin content, it deteriorated markedly in palatability. The boiled cabbage simmered for one hour scored 5.6 out of a possible 15.

Coldslaw: Prepared by Method 3, using either dressing, coldslaw scored about the same. All four lots of coldslaw prepared by Method 4, whether marinated for one-half hour or for two hours at either 85 to 92°F. or 32 to 35°F., rated about the same, from 11.6 to 13. The coldslaw (Method 4), which had been rehydrated for one-half hour in a larger proportion of water at 85 to 92°F., scored slightly higher than that which had been rehydrated for four hours in a smaller proportion of water at 32 to 35°F. (Method 3). Percentages of total solids were similar. The results of these two studies indicate that satisfactory coldslaw could be prepared in a warm climate without refrigeration if the rehydration time is short, the proportion of soaking water sufficiently large to allow for optimum rehydration, and the coldslaw served soon after marinating.

The four lots of coldslaw prepared by Method 4 were scored about the same as the cabbage boiled in the larger proportion of water (Method 2) and higher than the cabbage boiled by the other methods.

Comparison of Sulfited and Unsulfited Cabbage: On the dry basis, the unsulfited cabbage reported previously, Fenton *et al.* (1946), contained about half as much ascorbic acid, about four times as much thiamin, slightly more riboflavin, and about the same amount of niacin as the dehydrated, sulfited cabbage herein reported. The retention of ascorbic acid in the unsulfited cabbage that was cooked by the best of the five methods (minimum of water in the steam-jacketed kettle) was only 22 per cent, whereas the retention of ascorbic acid in boiled, sulfited cabbage even after simmering for one hour was 35 per cent.

Thiamin retention in the unsulfited cabbage ranged from 42 per cent in cabbage cooked in a large amount of water for 75 minutes to 82 per cent in cabbage cooked 12½ minutes in a small amount of water and held for three hours at 90°C.(194°F.); in the sulfited cabbage, thiamin retentions ranged from 29 per cent in the boiled cabbage that was cooked in a large proportion of water and then simmered for one hour to 61 per cent in the coldslaw prepared by Method 3. Riboflavin and niacin retentions were not affected greatly by sulfiting.

The unsulfited and sulfited cabbage received similar scores when prepared by similar methods. In general, the sulfited cabbage scored higher in color and texture because it retained its light, attractive, green color even after long cooking, and it was more tender than the unsulfited cabbage. It scored slightly lower than the unsulfited cabbage in flavor because of a weak cabbage flavor and the presence of a slight sulfur flavor.

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EFFECTS OF DIFFERENT METHODS OF DEHYDRATION ON VITAMIN AND MINERAL VALUE OF MEATS

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Although considerable data on the nutritive value of meat have appeared in the recent literature, most of them have been with reference to fresh meats, raw or cooked [Waisman and Elvehjem (1941); McIntire, Schweigert, and Elvehjem (1943); McIntire, Schweigert, Henderson, and Elvehjem (1943); Schweigert, McIntire, and Elvehjem (1943); Cover, McLaren, and Pearson (1944)]. Information on the composition of dehydrated meats with respect to vitamins and minerals is still relatively meager [Rice and Robinson (1944)].

As a part of the United States Department of Agriculture's intensive investigations of the development of practical methods for producing dehydrated meat of satisfactory quality, this study was planned to provide information concerning the thiamine, riboflavin, nicotinic acid, iron, and phosphorus content of dehydrated meats, and the changes occurring in these constituents during dehydration and storage.

The types of equipment, kinds of meat, preparation for processing, pretreatment and processing, as well as types of packaging and conditions of storage, have been described elsewhere.[1]

EXPERIMENTAL PROCEDURE

One series of experiments in this investigation consisted of a detailed study of pork and beef produced by two types of processes which were considered important because of their adaptability for commercial use—vacuum-rotary and kettle-cabinet methods.

Several different lots of meat were used and several processing runs were made for this purpose. Pork was the meat on which the greater emphasis was placed in the over-all investigation in meat dehydration because of the probability that more of that kind of meat will be dehydrated in the United States.

Since comparison of processes was the general theme of the research on dehydrated meat, another series of experiments was conducted using one large lot of pork which was subdivided for study of six different methods of dehydration. The processes selected for this purpose were those which had shown good possibilities in preliminary studies. The

[1] Other papers dealing with the Emergency Meat Dehydration Investigations of the United States Department of Agriculture have been published in previous issues of *Food Research*. Two appear as companions to the present paper and it is planned to publish still others in future issues of the journal. See also U. S. Dept. Agr. Circ. 706.

conditions of processing employed in each case were those which previous experience indicated to be most practicable. The processes consisted of the following: vacuum-rotary, kettle-air rotary, drum-air flotation, kettle-cabinet with and without antioxidant added to the meat prior to processing, and freezing-cabinet [U. S. Dept. Agr. Circ. 706 (1944)].

In the study of effect of storage, two lots of pork were used. One had been dehydrated by a commercial firm and the other in the Bureau of Animal Industry laboratories. Both were packaged in Container E, according to U. S. Dept. Agr. Circ. 706. This was an envelope-type, lead-foil bag which had been found to be most resistant to oxygen penetration and, perhaps, to be the best substitute for tin cans. The samples were stored at—17.8, 21.1, 43.3°C.(0, 70, 110°F.) and at temperatures alternating between 10 and 43.3°C.(50 and 110°F.) on a 24-hour cycle for eight, 24, and 36 weeks for the commercially dehydrated meat, and eight weeks for the experimentally processed sample. A constant relative humidity of 80 per cent was maintained at all storage temperatures except 0°F.

Unprocessed meat, freshly dehydrated, and stored dehydrated meats, both before and after cooking, were analyzed. The standard cooking procedure developed for palatability tests [U. S. Dept. Agr. Circ. 706 (1944)] was used for cooking the meats for analysis. The cooking liquid was included in the samples of meat analyzed.

Proper sampling is of particular importance in the analysis of meats, since the different parts of animals have been found to vary considerably in their content of vitamins and minerals. Therefore, a great effort was made to secure representative samples. About five pounds of meat was withdrawn at each stage of processing for these experiments. Both the raw and dehydrated meats were finely ground and thoroughly mixed prior to sampling. They were spread out in thin layers on large trays. Random samples were then taken, varying in size according to nutrient concentration with the type and form of meat, i.e., pork or beef, processed or unprocessed. For the unprocessed meats, 100- to 400-gram samples were used; for the dehydrated meats, 50 to 200 grams.

The cooked meats also were ground, the cooking juices being added and thoroughly blended. In these cases samples for analysis were taken from the resulting emulsions.

In preparing the samples for vitamin assay the meat samples were homogenized in Waring blendors in weighed amounts of extracting medium. Aliquots equivalent to three to 50 grams of the original meat were analyzed. The analytical methods used while this study was in progress (Sept. 1942 to May 1943) were as follows:

Thiamine was determined by the thiochrome procedure. Most of the pork samples were rich enough so that direct determinations could be made by the method of Hennessy and Cerecedo (1939), with modifications by Hennessy (1941). Where adsorption was necessary, the Conner and Straub (1941a) modification was employed. The enzymes, mylase or polydase, which were found to be more efficient than clarase for digestion of the meats, were used. Analyses were carried out on fat-free aliquots. For beef, where difficulties were encountered, the method of Harris and Wang (1941a, b) was tried.

Riboflavin was assayed chemically and microbiologically. To avoid the effects of the presence of fat, analyses were carried out on fat-free aliquots. The Strong and Carpenter (1942) modification of the original method of Snell and Strong (1939) was used for the microbiological method. Since riboflavin was determined chemically on a sample other than that used for thiamine, the Conner and Straub (1941b) combined determination was not used. However, the modifications of Hodson and Norris (1939), Ferrebee (1940), and Conner and Straub (1941a) were all necessary at some time during the analyses because of the presence of varying types of interfering substances.

Nicotinic acid was determined by the method of Snell and Wright (1941) as modified by Isbell (1942).

Standard curves were prepared with every set of vitamin assays. Recovery analyses for thiamine were also made on every run.

The o-phenanthroline method of Cowling and Benne (1942) was used for determining total iron.

Phosphorus and moisture were determined by the A.O.A.C. methods (1940).

RESULTS AND DISCUSSION

The data obtained in these experiments are summarized (Tables 1, 2, and 3). The nutrient content was calculated on the moisture-free, fat-free basis [2] because, although in preparing dehydrated meat it was aimed to prepare a product the moisture content of which should not exceed 10 per cent and the fat content of which should not exceed 30 per cent in the case of beef and 40 per cent for pork, according to adopted specifications, the different processed products were not exactly alike in this respect. Further, the analyses of the chemical composition of these products [2] have shown that varying losses of fat occurred, depending on the method of handling and processing. In general, the data showed a loss in fat. These observations emphasized the need of a uniform basis for calculating the data in order to make comparison possible.

Comparison of the chemical and biological methods for thiamine and the chemical, microbiological, and biological methods for riboflavin, using one lot of dehydrated pork, gave results in good agreement.

The data giving the vitamin and mineral contents of meats processed by different methods (Tables 1 and 2) indicate that during dehydration losses occurred in riboflavin, thiamine, and phosphorus in varying degrees. Thiamine being the most labile nutrient of the three, was affected to a greater degree than the other constituents.

The differences in values (Tables 1 and 2) may be attributed to a number of factors. One of these was the use of different lots of pork and of beef in the more extensive study of the vacuum-rotary and kettle-cabinet processes (Table 1). Another is the fact that in general, most of the pork used in these experiments consisted of ham, loin, picnic shoulder, shoulder butt, and lean trimmings. The beef consisted of chuck, rib, plate,

[2] The proximate composition of these products is reported in the paper "Chemical Composition of Raw and Dehydrated Meat" which also appears in this issue of *Food Research*.

TABLE 1

Effect of Processing on Vitamin and Mineral Value of Meat[1]

Method of processing	Kind of meat	Lots of meat		Nicotinic acid			Riboflavin			Thiamine			Phosphorus			Iron		
		Raw	Dehydrated	In raw meat	In dehydrated meat	Retention	In raw meat	In dehydrated meat	Retention	In raw meat	In dehydrated meat	Retention	In raw meat	In dehydrated meat	Retention	In raw meat	In dehydrated meat	Retention
				mg.	*mg.*	*pct.*	*mg.*	*mg.*	*pct.*	*mg.*	*mg.*	*pct.*	*mg.*	*mg.*	*pct.*	*mg.*	*mg.*	*pct.*
Vacuum-rotary—	Pork	7	11	23.9	23.8	101.2	1.66	1.42	88.1	3.95	2.18	60.1	1,010	990	98.6	10.3	10.2	108.3
Dehydrator A	Beef	2	4	24.4	17.7	81.8	1.22	0.87	76.4	.....	.....	.....	884	857	93.4	7.3	12.3	146.9
Kettle-cabinet—	Pork	11	11	17.6	13.7	82.7	3.54	2.72	83.4	4.01	1.73	45.1	10,260	862	87.5	8.7	7.7	87.1
Dryer A	Beef	2	2	19.8	17.9	92.7	2.85	2.57	90.7	.....	.....	.....	892	810	92.5	11.8	14.6	126.1

[1] Fat-free, moisture-free basis (100 gm.); average of the individual retentions for each lot processed, not calculated from the average content of the several lots.

TABLE 2

Comparison of Different Dehydration Processes With Respect to Vitamin and Mineral Value of Freshly Dehydrated Pork[1]

Dehydration process	Nicotinic acid	Riboflavin	Thiamine	Phosphorus	Iron	Retention				
						Nicotinic acid	Riboflavin	Thiamine	Phosphorus	Iron
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
None (fresh pork)[2]	14.5	3.97	3.55	1,020	7.9	.....	.....	.....	.....	.....
Freezing-cabinet	16.1	2.26	3.52	946	11.2	111.0	57.1	99.0	92.8	141.8
Vacuum-rotary	17.2	2.55	3.44	1,000	12.2	118.6	64.4	96.8	98.0	154.4
Kettle-air rotary	15.3	2.59	2.79	901	22.8	105.5	65.2	78.5	88.3	101.9
Drum-air flotation	14.8	2.76	2.36	923	8.9	102.1	69.6	66.3	90.5	112.6
Kettle-cabinet	13.5	2.43	2.31	961	8.9	93.1	62.3	65.0	94.2	112.6
Kettle-cabinet, antioxidant (0.03% gum guaiac) added	13.6	2.67	2.71	905	8.9	93.8	67.3	76.1	88.7	112.6

[1] Fat-free, moisture-free basis (100 gm.). [2] Pork from the same lot was used in every process.

loin, round, shank, rump, and flanks. These cuts were incorporated in the meat for processing in the proportions that result from the usual method of cutting up a carcass, but quantitative control so that each batch of meat studied contained each cut in the same proportion was not possible. Thus differences from lot to lot of meat were expected since different parts of the carcass vary greatly in their vitamin and mineral composition. Another factor which adds to the variability of the data is the extent to which the fat for certain experimental reasons was trimmed away from the meats. Differences purposely made in the precooking procedures, no doubt, are also responsible for the differences in the vitamin and mineral values. The composition of the dried product may likewise be influenced not only by the composition of the raw meat, but also by the extent of drying and any losses in constituents other than moisture that occur during processing. The vitamin contents may vary with (1) the type, age, and quality of the meat; (2) quantity of connective tissue present in the raw meat; (3) the maximum processing temperature; and (4) the duration of exposure to such temperature and probably, also, to air and light.

Furthermore, theoretically, the juices which seeped away from each charge of the ground or precooked meat during processing were returned to that particular batch of meat and dehydrated together with the meat. However, again, exact quantitative control was impossible and the original nutritive values could not be retained in full. Thus, differences from lot to lot of meat caused by this factor were inevitable.

Increases were found in the iron content of the dehydrated meats. This was probably due to contamination from the equipment used in processing the meats.

It is apparent, therefore, that losses associated with the handling of the meats and with the methods of processing that are variable and are unavoidable affect the results on the mineral and vitamin value of dehydrated meats.

The thiamine content of dehydrated beef is not reported. The initial low-thiamine potency of beef, especially after dehydration, made it practically impossible to obtain concordant results with this vitamin, because difficulties were encountered which were caused, apparently, by interfering substances. Similar experiences were described by Hinman, Halliday, and Brooks (1944).

In storage, as shown (Table 2), there was appreciable loss of thiamine, in pork after eight weeks at 70°F. and after longer periods of time even at 0°F. Severe losses were encountered at the higher temperatures. Nicotinic acid was not affected by eight weeks' storage; however, it appeared to be unstable at all temperatures after storage for 24 weeks or longer.

The analyses of the cooked dehydrated meats, fresh and stored, showed that losses occurred in thiamine only, and that they were approximately of the same magnitude as the losses found in cooking fresh meats. These cooking losses were caused by heat or oxidation only, since the cooking broth was included in the assay of the cooked meats.

The comparison study of the six different types of processes showed (Table 2) that there was very little if any loss of nicotinic acid; the values for the kettle-cabinet process, although somewhat lower than the others,

are within experimental error of the method. Varying but small losses of phosphorus were found in samples of pork dehydrated by each of the six methods. The iron content of the dehydrated meat showed increases in each case acquired, no doubt, from the equipment used in processing.

Riboflavin and thiamine were affected to the greatest degree. The riboflavin content, although noticeably reduced, was approximately as well retained in one process as in the others.

TABLE 3

Study of Storage Effects on Dehydrated Pork [1]

Origin of sample	Time of storage	Temperature of storage	Content		Retention	
			Nicotinic acid	Thiamine	Nicotinic acid	Thiamine
	wk.	*°F.*	*mg.*	*mg.*	*pct.*	*pct.*
Commercial firm [2]	8	0	27.9	1.690	.....	.....
		70	29.7	1.130	106.4	67.8
		110	29.4	0.007	105.3	0.4
	24	0	23.7	1.920	84.8	113.5
		70	23.2	0.880	82.9	51.9
		110	20.5	0.010	73.4	0.6
		110-150	21.1	0.005	75.4	0.3
	36	0	22.5	1.220	80.5	72.5
		70	21.3	0.440	76.2	26.1
		110	20.1	0.001	71.9	0.1
		110-150	23.1	0.002	82.8	0.1
Agricultural Research Center [2]	8	0	21.3	4.120	.....	.....
		70	21.3	3.000	0.0	72.1
		110	22.4	0.750	105.2	18.2
		110-150	22.3	1.440	104.7	35.2

[1] Fat-free, moisture-free basis (100 gm.). [2] Air packed in asphalt-impregnated kraft, 0.0005-inch lead foil, and 300 M.S.A.T. cellophane laminated together with the cellophane inside; two side seals.

Since thiamine is the most unstable of the vitamins in meat, being affected by heat, oxidation, or solution, and also because pork is richest in this vitamin, the processes were evaluated in this study with respect to conservation of thiamine in the finished products (Table 2). Retention of this nutrient was greatest in the samples of pork processed by the freezing-cabinet method. In this case, there was no precooking; the fresh meat was frozen overnight and then dried at 48.9°C.(120°F.). The meat produced by the vacuum-rotary method, where it was exposed to little air, also retained most of its thiamine, although the losses were slightly greater than in case of the freezing-cabinet process. The pork had undergone appreciable loss of the vitamin when prepared by the kettle-air rotary method where the meat was subjected to a high temperature and exposure to air. The samples dried by the air-flotation and kettle-cabinet processes, where they were exposed to large volumes of air and to comparatively high temperatures, showed poorest thiamine retention. The inclusion of an antioxidant in the form of 0.03 per cent gum guaiac in the kettle-cabinet process improved this process by preventing to a certain degree the destruction of thiamine.

SUMMARY

The effect of different types of dehydrating processes on the thiamine (pork only), riboflavin, nicotinic acid, iron, and phosphorus contents of pork and beef was determined. Thiamine was found to be the most unstable of the nutrients during processing, the degree of retention varying with the type of method employed. The freezing-cabinet and vacuum-rotary methods were the most efficient in conserving the thiamine content of pork. Addition of an antioxidant to pork dehydrated by the kettle-cabinet process partially prevented destruction of thiamine. No important changes in any of the other nutrients were brought about by the processes studied.

Storage losses were extensive in case of thiamine at temperatures above 70°F. even after eight weeks.

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NUTRITIVE VALUE OF PROTEIN IN DEHYDRATED MEAT[1]

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Dehydrated meat is essentially a high-protein food. Extensive information is available concerning the nutritive properties of the protein in meats and meat products in general, but comparatively little has been reported concerning dehydrated meat. The purpose of the experiments herein reported was to determine the digestibility and biological value[2] of the protein in pork, beef, and mutton dehydrated by various methods, and the effects of storage on these values.

Poling, Schultz, and Robinson (1944) determined the effects of dehydration, canning, roasting, and frying on the nutritive value of the protein in beef and pork. They concluded that the proteins of dehydrated pork and fried fresh pork shoulder were slightly superior in nutritive value to those in canned cured pork shoulder, roast fresh pork shoulder, and dehydrated beef.

EXPERIMENTAL PROCEDURE

The nutritive values of the protein in a number of lots of dehydrated beef, pork, and mutton were determined by feeding a diet containing 10 per cent of meat protein. In addition the diet contained either two per cent of a concentrate from brewers' yeast, or high potency brewers' yeast itself, as a source of the B vitamins. Vitamins A and D were supplied in two per cent of cod-liver oil. Sufficient fat of the same kind as the dehydrated meat was added to make 10 per cent. Four per cent of salt mixture and sufficient dextrin to make 100 per cent completed the diet.

Each diet was fed for 30 days to eight young male albino rats weighing approximately 40 grams each at the beginning of the test. Rats from each litter were distributed among the several groups on test at one time. Each rat was kept in an individual cage with a raised screen bottom; it was supplied with water and with feed in a self-feeder. The rats were weighed twice weekly and the feed consumption of each was recorded, but only the average values for each group are shown. The temperature of the rat laboratory was maintained at approximately 23.9°C.(75°F.).

The apparent digestibility of the total protein was determined as follows: After a feeding test had been in progress about 23 days, all of the feces from each rat in a group were collected for seven days, dried at

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[1] This paper deals with the Emergency Meat Dehydration Investigations of the United States Department of Agriculture, conducted in response to wartime needs. Other papers in the series appear in this issue of *Food Research*; still others have been published in earlier issues of the journal.

[2] The term "biological value" as used in this paper denotes the value of the total crude protein for maintenance and growth in male albino rats, expressed as gain in weight per gram of protein consumed.

100°C.(212°F.) to constant weight, and analyzed for nitrogen. Hair was separated from the dried feces by a blast of air. The feed consumed during the seven-day test was weighed. The nitrogen content of the diet was determined and the apparent digestibility of total protein was calculated.

The true digestibility of the protein in some of the samples of dehydrated meat was determined as follows: The digestion test was conducted as outlined above, but at the end of the 30-day feeding test each rat was changed to a diet containing only about one per cent of crude protein in the yeast or yeast concentrate. In a few cases sufficient dried whole eggs to furnish four per cent of protein also were added. After the rats had eaten this diet for three days, feces were collected for seven days and the quantity of feed consumed during the same period was recorded. The feces were dried, weighed, and analyzed for nitrogen. The nitrogen excreted during this period, so-called "metabolic nitrogen," together with the data for previous digestion tests with the same rats, were used in calculating the true digestibility of the protein in the dehydrated meat. Correction was made for the difference in feed intake by each rat during the two tests.

DESCRIPTION OF SAMPLES

A total of 38 samples of dehydrated meat and one each of dried skim milk and of dried eggs were tested. Twenty-four of the samples of dehydrated meat were prepared in the meats laboratory of the Bureau of Animal Industry at Beltsville, Md. Fourteen samples of dehydrated meat and one each of dried eggs and of dried skim milk were obtained from commercial sources. All samples were analyzed chemically before being used in making up rations. Lack of space, and in some cases lack of information, does not permit a detailed description of the procedure followed in the preparation of each lot of dehydrated meat; the general procedure employed is described briefly (Table 1).

EXPERIMENTAL RESULTS

The biological values and the digestive coefficients of the protein in 16 lots of dehydrated pork (Table 2) should be compared with the data (Table 1) in which the method of dehydration is described. For the most part, the biological values of the several lots of pork did not differ greatly. The first six lots of pork, all prepared by the vacuum-rotary process, had values ranging from 3.37 to 3.72 gm. gain per gram of protein consumed, with an average of 3.51 gm. The apparent digestive coefficients of these samples were very similar.

The following eight samples of pork, with the exception of Sample 4150, had biological values ranging from 3.11 to 3.58 gm. with an average of 3.35 gm. Commercial Sample 4150, method of preparation unknown, had a much lower value than the other samples. Most of the samples in this group were dehydrated by the cabinet method, but the methods of precooking varied.

The last two samples were commercially canned spiced ham and canned pork luncheon meat which were dehydrated in the Bureau laboratory. The average biological value for these products was 3.22 gm. which is considerably lower than the average value for the first six lots of pork, and

TABLE 1

Procedure Followed in Dehydration of Pork, Beef, and Mutton Used in Studies of Nutritive Value of Protein

Sample No.	Kind of meat	Precooking			Dehydration		
		Type of cooker	Time	Temperature	Type of cooker	Time	Temperature
			min.	*°F.*		*hr., min.*	*°F.*
4167	Pork	Vacuum-rotary [1]	20	190-212	Vacuum-rotary	1-30	148-196
4168	Pork	Vacuum-rotary	75	212	Vacuum-rotary	4-0	128-150
4169	Pork	Vacuum-rotary	85	165	Vacuum-rotary	7-0	100-148
4184	Pork	Vacuum-rotary	70	212	Vacuum-rotary	5-30	125-150
4185	Pork	Vacuum-rotary	70	165	Vacuum-rotary	6-0	90-153
4203	Pork	Vacuum-rotary	70	212	Vacuum-rotary	3-45	140-168
4208	Pork	Double-drum	0.75	315	Cabinet	4-0	200
4224	Pork	Kettle	45	212	Cabinet	3-0	180
4225	Pork	Kettle	45	212	Cabinet	5-0	180
4226	Pork	Double-drum	0.75	315	Cabinet	5-0	180
4229	Pork	Retort	60	245	Cabinet	2-45	180
4150	Pork	.....	....	.....	.....	.....	.....
4164	Pork	Kettle	80	165	Air-rotary	2-0	120-350
4163	Pork	.....	....	.....	.....	.....	.....
4154	Pork [2]	Retort	....	.....	Cabinet	20-0	140
4155	Pork [3]	Retort	....	.....	Cabinet	20-0	140
4157	Beef	Vacuum-rotary	50	190	Vacuum-rotary	9-30	100-175
4158	Beef	Vacuum-rotary	50	190	Vacuum-rotary	2-0	160
4165	Beef	Vacuum-rotary	70	212	Vacuum-rotary	3-30	120-150
4166	Beef	Vacuum-rotary	70	165	Vacuum-rotary	6-0	109-150
4188	Beef	Vacuum-rotary	70	165	Vacuum-rotary	5-0	105-155
4127	Beef	Double-drum	0.75	320	Cabinet	2-15	180
4135	Beef	Not cooked	....	.....	Cabinet	20-0	140
4156	Beef	Double-drum	1.08	337	Cabinet	4-0	160
4227	Beef	Kettle	70	212	Cabinet	1-30	180
4228	Beef	Kettle	70	212	Cabinet	3-0	180
4142	Beef	Vacuum-rotary	40	170-175	Cabinet	1-20	140-170
4130	Beef	.....	....	.....	.....	.....	.....
4131	Beef	.....	....	.....	.....	.....	.....
4132	Beef	.....	....	.....	.....	.....	.....
4143	Beef	.....	....	.....	.....	.....	.....
4144	Beef	.....	....	.....	.....	.....	.....
4146	Beef	Kettle	12	212	Vacuum	17-0	200
4148	Beef [4]	Retort	....	.....	Cabinet	20-0	140
4205	Mutton	Vacuum-rotary	70	212	Vacuum-rotary	4-0	150-160
4204	Mutton	Double-drum	0.75	315	Cabinet	6-30	180
4206	Mutton	Kettle	95	212	Cabinet	3-0	180
4207	Mutton	Kettle	95	212	Cabinet	4-0	150

[1] In all instances when the vacuum-rotary machine was used for precooking, it was operated without vacuum. [2] Canned spiced ham dehydrated in laboratory. [3] Canned pork luncheon meat dehydrated in laboratory. [4] Canned corned beef dehydrated in laboratory.

slightly lower than the average for the second group of seven samples. It is noteworthy that Poling, Schultz, and Robinson (1944) also found that dehydrated pork had a slightly higher nutritive value than canned pork.

DEHYDRATED BEEF

Results on the nutritive properties of the protein in 18 lots of beef and in one lot each of dried skim milk and of dried eggs are shown (Table 3).

TABLE 2

Average Biological Values and Digestive Coefficients of Protein in Dehydrated Pork as Determined by Rat-Feeding Tests

Sample No.	Gain in 30 days		Digestibility of protein	
	Total	Per gram of protein	Apparent	True
	gm.	*gm.*	*pct.*	*pct.*
4167	120	3.62	91.4	.....
4168	111	3.35	91.9	.....
4169	99	3.43	93.2	.....
4184	122	3.54	92.6	.....
4185	114	3.72	92.9	.....
4203	101	3.37	91.9	.....
4208	102	3.37	91.7	.....
4224	106	3.47	.....	.....
4225	90	3.11	.....	.....
4226	100	3.28	.....	.....
4229	100	3.31	.....	.....
4150	69	2.68	.....	.....
4164	101	3.34	.....	.....
4163	119	3.58	.....	.....
4154	94	3.16	91.6	98.8
4155	100	3.27	90.2	97.6
Average	103	3.35	92.0	98.2

TABLE 3

Average Biological Values and Digestive Coefficients of Protein in Dehydrated Beef as Determined by Rat-Feeding Tests

Sample No.	Gain in 30 days		Digestibility of protein	
	Total	Per gram of protein	Apparent	True
	gm.	*gm.*	*pct.*	*pct.*
4157	89	3.00	.....	.....
4158	97	3.18	.....	.....
4165	102	3.30	.....	.....
4166	110	3.49	.....	.....
4188	105	3.47	.....	.....
4127	90	3.26	91.4	99.8
4135	107	3.63	91.0	99.7
4156	101	3.22	.....	.....
4227	93	3.01	.....	.....
4228	88	3.05	.....	.....
4142	100	3.34	89.9	.....
4130	66	2.66	88.9	97.6
4131	91	3.11	91.2	99.3
4132	77	2.87	.....	.....
4143	84	3.12	85.9	94.9
4144	89	3.15	85.9	95.1
4146	92	3.33	91.0	98.9
4148[1]	54	2.42	83.3	91.6
Average	91	3.15	88.7	97.1
4133[2]	95	3.15	80.3	89.9
4149[3]	124	4.28	89.4	98.2

[1] Canned corned beef dehydrated in laboratory. [2] Dried skim milk. [3] Dried eggs.

There was considerable variation in the biological values for the different lots of beef. Sample 4135 with the highest value was uncooked beef round which was dehydrated at 60°C.(140°F.). Two commercial samples, 4130 and 4132, had relatively low values, but information concerning the preparation of these products was lacking. Sample 4148, canned corned beef, was dehydrated at 140°F. This product had the lowest biological value of any of the samples of beef. With the exception of the samples noted above, the biological values ranged from 3.00 to 3.49 gm. gain per gram of protein consumed, with an average of 3.22 gm.

The protein in dried skim milk had the same biological value as the average for all the lots of beef, but that in dried eggs had a much higher value.

TABLE 4

Average Biological Values and Digestive Coefficients of Protein in Dehydrated Mutton as Determined by Rat-Feeding Tests

Sample No.	Gain in 30 days		Apparent digestibility
	Total	Per gram of protein	
	gm.	*gm.*	*pct.*
4205	99	3.22	90.9
4204	86	3.16	90.5
4206	93	3.19	90.7
4207	102	3.24	91.5
Average	95	3.20	90.9

The digestibility of the protein in the different lots of beef varied considerably. The apparent digestive coefficients ranged from 83 to 91 per cent, and the true digestive coefficients from 92 to 100 per cent. The average digestibility of the protein in all lots of beef was considerably higher than that for dried skim milk and approximately the same as that for dried eggs.

DEHYDRATED MUTTON

Results on the nutritive properties of the protein in four lots of dehydrated mutton (Table 4) show that the biological values and the apparent digestive coefficients ranged between comparatively narrow limits. The average values for all samples of mutton are very similar to those reported for pork (Table 2) and for beef (Table 3).

EFFECTS OF STORAGE ON PROTEIN AND FAT IN DEHYDRATED PORK

A few experiments were conducted to determine the effects of storage under different conditions on the nutritive properties of the protein in dehydrated pork. The meat was packed in laminated lead-foil [3] cartons with a capacity of about one pound. All samples were obtained from the same original lot of pork. Results of the tests are shown (Table 5). The biological value of the protein in Sample 4176 appears too low since it is lower than the value for Sample 4199 which was stored for a much longer

[3] Asphalt-impregnated kraft, 0.0005-inch lead foil, and 300 M.S.A.T. cellophane laminated together with the cellophane inside; two side seals.

time at the same temperature. If this value be excepted, it appears that the biological value of the protein was appreciably affected by the time and temperature of storage.

TABLE 5

Effects of Storage on Nutritive Properties of Protein in Dehydrated Pork as Determined by Rat-Feeding Tests

Sample No.	Storage conditions		Gain in 30 days		Apparent digestibility
	Time	Temperature	Total	Per gram of protein	
	days	*°F.*	*gm.*	*gm.*	*pct.*
4164	0	....	101	3.34	92.1
4176	56	0	78	2.95	89.5
4178	56	70	95	3.19	91.2
4179	56	110	86	3.12	91.0
4199	112	0	97	3.25	91.0
4200	112	70	94	3.18	91.4
4201	112	110	75	2.94	90.6

An experiment was also conducted to determine the effects of storage on digestibility of the fat in dehydrated pork. The pork was packed in the same kind of a container as in the previous experiment and stored for 168 days at different temperatures. The test rations were made up to contain 15 per cent of fat in dehydrated pork and the rations were adequate in other respects. The procedure followed in the digestion tests was similar to that previously described by Hoagland and Snider (1940). The results of the tests are shown (Table 6); digestive coefficients did not differ materially and they are similar to values previously reported for lard by the above-mentioned writers (1940).

TABLE 6

Effects of Storage on Digestibility of Fat in Dehydrated Pork as Determined by Rat-Feeding Tests

Sample No.	Storage conditions		True digestibility
	Time	Temperature	
	days	*°F.*	*pct.*
4221	168	0	97.2
4222	168	70	96.4
4223	168	110	96.6

SUMMARY

The biological value and digestibility of the protein in dehydrated pork, beef, and mutton were determined by experiments with young male albino rats. The term "biological value" denotes the value of the total crude protein for maintenance and growth. The products tested included 16 lots of pork, 18 lots of beef, and four lots of mutton. One lot each of dried skim milk and of dried eggs were tested for comparison. The experiments were conducted at the Beltsville Research Center of the United States Department of Agriculture, Beltsville, Maryland.

The biological values of the protein in individual lots of pork and beef were found to differ materially, but the values for mutton were relatively uniform. The average values for all lots of pork, beef, and mutton, respectively, were similar. The protein in dried skim milk had approximately the same biological value as the average values for dehydrated meat, but dried eggs had a considerably higher value.

The digestibility of the protein in individual lots of pork and mutton differed only slightly, but the values for some lots of beef differed materially. The average digestibility of the protein in pork, beef, and mutton, respectively, was higher than that of dried skim milk, and similar to that of dried eggs.

The storage of dehydrated pork for 168 days at —17.8, 21.1, and 43.3°C. (0, 70, and 110°F.) was without material effect on the digestibility of the fat.

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CHEMICAL COMPOSITION OF RAW AND DEHYDRATED MEAT[1]

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The chemical composition of dehydrated meat is a matter of basic importance. To insure good keeping quality the moisture content must be given most careful attention, and the proportions of protein, fat, and ash have broad meaning in relation to quality or nutritive value or both.

Excluding very young animals there is little variation in the gross chemical composition of fat-free muscle from cattle, hogs, and sheep. It is when fat has been deposited, to a greater or lesser extent, within the muscle tissue that important variations in the proportions of moisture, protein, fat, and ash begin to appear. This situation is rapidly accentuated when total edible meat, instead of muscle, is the basis of consideration and external, internal, and intermuscular fats become a factor.

Hence there are at least two factors which affect the composition of the raw material used in meat dehydration: one is the fatness of the animal or carcass selected; the other is the extent to which fatty tissue is trimmed away from the lean tissue. The processor can drastically influence the composition by attention to these two factors. Ordinarily the lower grade or leaner sort of animal is used and trimming is held to a minimum on account of the expense involved. A typical analysis of fresh pork ready for drying is as follows: moisture 65.3, protein 18.8, fat (ether extract) 15.0, and ash 0.9 per cent. When such meat is dehydrated to 10 per cent moisture content and with no loss of other constituents in the processing, its calculated composition in the dehydrated state with respect to protein, fat, and ash is about 48.8, 38.9, and 2.3 per cent, respectively.

As indicated, the chemical composition of the finished product is influenced by composition of the raw material, extent of the drying, and any losses in constituents in addition to moisture that occur during the processing. Assuming that the composition of the raw material and the moisture content of the dehydrated meat can be controlled, the problem becomes one

[1] This paper deals with the Emergency Meat Dehydration Investigations of the United States Department of Agriculture. Other papers in the series appear in this issue of *Food Research*; still others have been published in earlier issues of the journal.

[2] Formerly with the Agricultural Research Administration, U. S. Department of Agriculture, employed for emergency work on meat dehydration investigations.

relating to possible losses in protein, fat, or ash. So long as no juice is discarded and the meat is not overheated, it should be possible to retain all of the protein and ash. However, there are possibilities for loss of fat, associated with the method of processing and handling.

PROCESSES AFFECT COMPOSITION

Several of the processes involved in the emergency meat dehydration investigations were used for relatively large numbers of lots of pork. It was, therefore, feasible to make a study of the chemical composition of the finished product as affected by those processes. The mean composition of each of five representative groups of raw-meat samples processed by the vacuum-rotary, kettle-cabinet, drum-air flotation, kettle-air rotary, and drum-cabinet methods [3] are shown (Table 1). Further data show the differences in protein, fat, and ash between the theoretical proportions of those constituents in the dehydrated product, calculated from the raw material, and the actual proportions found. In calculation of the theoretical proportions a moisture content was assumed identical with that occurring in the lot of dehydrated meat produced. The mean moisture values for the five groups of dehydrated samples varied through the narrow range of 6.53 to 9.70 per cent.

TABLE 1

Effect of Certain Processes as Shown by Differences Between Calculated and Actual Composition of Dehydrated Pork [1]

General process of meat dehydration	Number of lots	Chemical composition of raw meat				Difference between calculated and actual composition of dehydrated meat		
		Water	Protein	Fat	Ash	Protein (gain or loss)	Fat (gain or loss)	Ash (gain or loss)
		pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Vacuum-rotary	30	67.73	19.44	11.84	1.01	+3.45	—2.97	+0.02
Kettle-cabinet	25	67.78	19.39	11.92	1.00	+4.53	—4.67	+0.10
Drum-air flotation	30	67.83	19.07	11.96	0.99	+11.16	—11.02	+0.38
Kettle-air rotary	14	64.61	18.99	15.04	0.98	+6.73	—5.83	+0.20
Drum-cabinet	30	68.85	19.99	10.07	1.01	+2.41	—1.55	—0.02

[1] Methods adopted by the Association of Official Agricultural Chemists were used in making all determinations.

It will be noted (Table 1) that essentially the situation was one of fat decrease counterbalanced by an increase in protein or in both protein and ash. Concerning the fat losses, there are two possibilities. One has to do with sampling and is associated with the fact that in the warm product the fat has a constant tendency to run to the bottom. Unless unusual and

[3] These processes are described in the following previously published papers:

Noel, W. A., Gray, W. E., Hankins, O. G., and Hollingshead, R. S., 1945. Processing equipment used in emergency meat dehydration research by United States Department of Agriculture. Food Research 10, 379-391.

Dunker, C. F., Hankins, O. G., and Bennett, O. L., 1945. Producing dehydrated meats by vacuum-rotary, plate-vacuum, and kettle-cabinet methods. Food Research 10, 445-456.

Hankins, O. G., Dunker, C. F., and Bennett, O. L., 1946. Producing dehydrated meats by drum-cabinet, retort-cabinet, and other methods. Food Research 11, 1-13.

persistent precautions are taken, samples are likely to be obtained that are slightly low in fat and high in protein and ash. The other possibility is related to the process itself, in that melted fat may become separated from the drying meat, and not be reincorporated with the product.

The nature of the vacuum-rotary equipment and process is such that there appears to be no opportunity for actual loss of fat. Therefore, the difference of 2.97 per cent between the calculated and actual values for the meat dehydrated by that process would seem to represent fat remaining in the machine or lost in removing the dehydrated product from the machine or difficulty in obtaining strictly representative samples.

The values of —4.67 and —1.55 per cent for the kettle-cabinet and drum-cabinet processes, respectively, indicate there was more loss in kettle than in drum precooking. If small quantities of melted fat were left in the bottom of the kettle when the cooked meat was removed for drying, this difference would be accounted for. The indicated loss in the case of the drum-cabinet process is particularly low.

The kettle-air rotary lots were dried in air-rotary Dehydrator A.[4] Comparison of the values of —5.83 and —4.67 per cent indicates that there was more fat loss in air-rotary Dehydrator A than in the cabinet. Apparently there was more accumulation of fat in the rotating cylinder of the former than drip loss, which must have been very small, in the case of the latter.

The drum-air flotation process was represented by a fat loss of 11.02 per cent—the greatest of the five processes considered. Comparison with the drum-cabinet process shows that most of the difference between the calculated and actual composition values was brought about during the flotation drying period and not during the drum cooking period. That considerable loss was occurring was evident from the fact that fat ran down the inside wall of the flotation chamber while the drying was in progress.

Attention is called to the fact that this work was done with laboratory or pilot-plant equipment. The results relating to actual, or even relative, fat losses associated with the different processes are not necessarily indicative of what should be expected in plants of commercial size. However, they do make clear that losses are likely to occur and suggest certain precautions that the processor may well take.

ELIMINATION OF SURPLUS FAT

Under usual conditions, trimming fat from the lean raw meat is a relatively expensive step in the processing. For this reason, it often appears essential to keep the trimming strictly to a minimum. Processors confronted with such a situation are deeply interested in less costly procedures for eliminating surplus fat, that may be employed as a part of the process itself or used as a final step following the actual drying. The foregoing discussion of differences between calculated and actual composition suggests means by which some fat can be eliminated during the processing

[4] This dehydrator and other equipment used in the investigations were described in the first paper in the series, published in the September-October, 1945, issue of *Food Research*.

Other methods are to collect the combination of juice and fat that comes from the meat in kettle and retort precooking, separate the fat from the juice, and add only the juice back to the meat. Possibilities for removing surplus fat after the drying is completed are draining with or without suction, pressing, and using the expeller type of machine involving continuous operation. Any method used must of course be under controlled conditions with respect to temperature and other factors.

COMPOSITION IN RELATION TO SPECIFICATIONS

It is apparent from the foregoing discussion that the chemical composition of dehydrated meat may be largely controlled. In fact, composition is predetermined to a great extent by the moisture and fat specifications that are employed. For example, the specifications adopted by the U. S. Department of Agriculture for use in the purchase of dehydrated beef and pork primarily for lend-lease purposes called for not more than 10 per cent moisture and 30 per cent fat in the case of the former product and not more than 10 per cent moisture and 40 per cent fat in the case of the latter. As much as 3.5 per cent of sodium chloride was permitted in the dehydrated beef, but the pork specifications prepared later did not provide for the addition of any of that ingredient.

Keeping within safe limits, it is only natural that the processor should be interested in approaching closely the maximum moisture content allowed. Moreover, with fat normally less expensive than protein, he is likely to have all of the fat in the dehydrated product that is permitted under the limit set by the purchaser. The same is true, of course, with respect to sodium chloride content. The difference between the sum of the percentages for the constituents specified—moisture and fat or moisture, fat, and sodium chloride—and 100 per cent is divided between protein and ash. The ratio of these two components is moderately constant among various lots of meat when the meat is not overheated and there is no loss of juice in the processing. The mean ratios of protein to ash for representative lots of dehydrated beef and pork produced by the Department of Agriculture were 21.2:1 and 20.4:1, respectively.

RELATED CHANGES IN COMPOSITION

The chemical composition of 30 lots of pork dehydrated by the Department of Agriculture is shown (Table 2). These lots were chosen to represent a narrow range of moisture content and are listed in the increasing order of fat content, which covers a wide range. The purpose, in part, of presenting this table is to give a clearer conception of the approximate variations in protein and ash that are associated with a wide variation in fat content. Moreover, it brings out clearly that as the latter increased, both protein and ash content decreased. The decrease in protein, obviously, accounted for most of the increase in fat. It is apparent, therefore, that if a primary objective in using dehydrated meat in the diet is to furnish a large proportion of the protein requirement, a product of high fat content will tend to defeat that purpose.

The chemical composition of a number of samples of dehydrated beef is shown in Table 3. As in the case of the pork samples (Table 2), they

TABLE 2

Chemical Composition of 30 Samples of Dehydrated Pork Listed in Increasing Order of Fat Content

Water	Protein	Fat	Ash
pct.	*pct.*	*pct.*	*pct.*
9.10	67.00	20.36	3.22
9.08	65.52	21.94	3.40
9.32	65.30	22.85	3.22
9.05	63.28	23.63	3.10
9.30	64.72	23.94	3.29
8.82	64.14	24.40	3.20
8.68	63.14	24.45	3.05
8.64	65.23	24.48	3.29
9.43	63.17	24.51	3.24
9.01	62.17	25.83	2.94
8.91	61.69	25.90	3.08
9.07	62.69	26.68	3.14
8.52	62.72	26.79	3.08
9.34	60.61	27.26	3.04
8.86	57.79	28.91	2.78
9.20	59.72	29.13	2.82
9.35	58.41	29.47	2.91
9.28	57.21	30.94	2.90
8.83	57.94	31.17	2.39
8.57	57.50	31.42	2.88
9.46	56.96	31.47	2.73
9.36	55.60	31.66	3.03
8,85	57.28	32.05	2.72
9.28	57.20	32.12	2.72
8.58	56.24	33.47	2.72
9.19	55.31	33.95	2.98
8.60	51.93	37.34	2.50
8.72	48.74	40.12	2.38
8.86	42.86	46.89	2.06
8.88	42.12	46.94	2.02

TABLE 3

Chemical Composition of 12 Samples of Dehydrated Beef Listed in Increasing Order of Fat Content

Water	Protein	Fat	Ash
pct.	*pct.*	*pct.*	*pct.*
8.65	73.14	14.85	3.48
8.67	70.71	16.57	3.47
9.24	70.13	17.03	3.26
8.58	70.03	18.45	3.28
9.23	65.01	23.02	3.11
9.38	60.69	26.49	2.87
8.88	59.24	28.73	2.85
9.44	58.22	28.82	2.65
8.59	57.45	30.00	2.72
8.84	54.83	33.29	2.64
8.99	53.95	34.10	2.43
8.95	47.68	40.87	2.32

were selected for practical uniformity in moisture and wide variation in fat content. It would be possible, of course, to produce dehydrated beef of higher fat content and correspondingly lower proportions of protein and ash, but that was not done in the research of the Department.

SUMMARY

Little variation occurs in the gross chemical composition of fat-free muscle from cattle, hogs, and sheep. However, when fat has been deposited by the animal, to a greater or lesser extent, important variations in the proportions of moisture, protein, fat, and ash of the total edible meat begin to appear.

The composition of the raw material used in dehydration is greatly affected by the fatness of the carcasses selected and the extent to which fatty tissue is trimmed away from the lean tissue.

The composition of dehydrated meat is influenced by composition of the raw material, extent of moisture removal, and any losses in other constituents that occur in the processing. Although there are possibilities for fat losses, all protein and ash should be retained in the processing if no juice is discarded and the meat is not overheated.

The difference between the calculated and actual composition of dehydrated pork showed the greatest fat loss to occur in the case of processing by the drum-air flotation method. The kettle-air rotary, kettle-cabinet, vacuum-rotary, and drum-cabinet methods followed in decreasing order. Although laboratory or pilot-plant equipment was used, the results indicated that losses may occur under commercial conditions and suggested precautions to avoid them. They also suggested means for eliminating surplus fat economically.

The specifications employed for moisture and fat content largely predetermine the chemical composition of dehydrated meat. The ratio of protein to ash is moderately constant.

Among dehydrated-meat samples a large increase in fat was mainly accounted for by decrease in protein content. Ash also was a compensating factor, but the decrease was small.

RETENTION OF ASCORBIC ACID IN RASPBERRIES DURING FREEZING, FROZEN STORAGE, PURÉEING, AND MANUFACTURE INTO VELVA FRUIT

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Among fruits preserved commercially by freezing in the United States, raspberries occupy an important position. Each year 15 to 20 million pounds are frozen, and in prewar years only strawberries exceeded raspberries in frozen tonnage. About 85 per cent of this tonnage is frozen in the Pacific Northwest.

Fresh raspberries are known to be rich in ascorbic acid. Todhunter and Robbins (1941) found excellent retention of ascorbic acid in red raspberries frozen in the Pacific Northwest. Packs frozen without sugar in enameled cans showed no loss during nine months' storage at —18°C. (0°F.). Dernovskaya-Zelentsova and Dylevskaya (1941) found 80 to 97 per cent of the ascorbic acid in raspberries retained during freezing at —8°C.(18°F.). Scheunert and Reschke (1939) reported that ascorbic acid did not decrease materially in raspberries during freezing or storage for one year. Antonov and Svereva (1940) found 60 to 73 per cent of the ascorbic acid in raspberries retained during freezing at —27°C.(—17°F.) and storage for six to eight months at —18°C.(0°F.). In 50-per cent sugar sirup the retention was 77 to 84 per cent, and with dry sugar 84 per cent.

This paper reports studies on raspberries frozen according to present commercial practice, including use of water-vapor-resistant cellophane liners and cardboard containers. Retention of ascorbic acid with and without sugar and during freezing and short storage, extended storage, defrosting, puréeing, flash-heating, and manufacture into Velva Fruit has been measured. The berries studied were partly from the principal raspberry area of the Pacific Northwest and partly from areas adjacent to the Western Regional Research Laboratory.

ANALYTICAL METHODS

The packages were usually defrosted in four to six hours at room temperature. When the samples reached room temperature, the drained weights were determined after draining for two minutes over an eight-mesh stainless-steel sieve. Duplicate 50-gram samples of both sirup and drained berries were used for the ascorbic-acid analysis. The amount of ascorbic acid per 100 grams of packaged fruit was then calculated from the amounts in the juice and drained berries. The determinations of ascorbic acid were performed according to the method of Loeffler and Ponting (1942). Either one per cent of metaphosphoric acid or 0.25 per

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cent of oxalic acid was used for extractions. Ponting (1943) pointed out the usefulness of oxalic acid for this purpose. The figures given in the tables are usually the average of determinations on four individual, one-pound packages. A high degree of uniformity of fruit was achieved by careful selection so that the range of values for the packages of an individual code was usually of the order of one mg. per 100 grams. All results are reported on the wet basis.

Berry extracts fade slightly in the photoelectric colorimeter in a manner comparable to fading caused by interfering substances in vegetable extracts. The blank calibration tube undergoes the same slight fading, however, when the berry extract is diluted with water rather than dye. The fading, therefore, is not due to a slow reaction between the dye and interfering substances; it appears to be due to a change in degree of dissociation, which in turn changes the intensity of color of the anthocyanin pigments during the dilution whether by dye or by water. More accurate results are obtained with a lapse of 30 seconds between dilution of blank tube and calibration of instrument, and between addition of dye and reading of unknown. The pH was determined with a glass-electrode meter. Acidity was determined by electrometric titration to pH 8 with the same instrument.

TABLE 1

Effect of Freezing and Two Months of Frozen Storage on Ascorbic Acid Content of Raspberries

Liquid added (3+1)	Ascorbic acid (mg. per 100 gm.)					
	Before freezing	After freezing		After 2 months' storage		
		In blend	Corrected for added liquid	In blend	Corrected for added liquid	Total loss
Dry (no sirup)	19	19	19	19	19	0
Water (no sirup)	18	12	16	12	16	2
39.5% sirup	18	13	17	13	17	1
50.5% sirup	16	12	16	12	16	0
68.0% sirup	17	12	16	12	16	1

RETENTION OF ASCORBIC ACID DURING FREEZING AND SHORT STORAGE

A lot of fresh berries, of uniform maturity and quality but unknown variety, was used for this experiment (Table 1). The berries were divided into five portions of at least 1,100 grams each. Small amounts (200 grams each) were packed in commercial, water-vapor-resistant, cellophane liners and waxed cardboard boxes. One part of water or sirup was added to three parts of fruit at the time of packaging and before freezing; three different concentrations of sirup were used. A portion was frozen in the field boxes and packaged after freezing. Freezing was accomplished in moving air at —23°C.(—10°F.).

A representative 100-gram sample of each portion was tested for ascorbic acid before freezing. Two packages were tested within one week after freezing and four packages after two months' storage at —23°C.

The frozen 200-gram packages were not defrosted in the open but were split approximately in half and each half was defrosted, blended, and extracted directly in 650 ml. of acid extractant. The filtrates were then mixed to compensate for any uneven splitting of the package. Sampling error was thus reduced to a minimum by the very large samples.

The results show that no significant loss occurred during the freezing process or storage for two months in berries frozen without sugar or with concentrations of sugar sirup varying from 40 to 68 per cent. A loss of about 10 per cent occurred in those to which water was added.

EFFECT ON ASCORBIC ACID OF CONDITION OF FRUIT, EXTENDED STORAGE, AND DEFROSTING

Experimental packs of Cuthbert raspberries, prepared according to commercial practice in a large commercial freezing plant at Gresham, Oregon, were used (Table 2). Here the berries are picked during the morning and brought to the plant during the early afternoon. They are ordinarily processed in the plant during the afternoon and evening. The processing includes thorough washing by immersion, drying and separation of extraneous matter on vibrating slat shakers, sorting on inspection belts prior to packaging in water-vapor-resistant cellophane liners and waxed cardboard boxes, and freezing in a cold-air blast. One part sirup is added to three parts of fruit by weight.

The most likely causes of defects in this commercial processing are delay before freezing and overmaturity of berries. Delay was simulated by allowing the berries to stand in the field boxes on the covered loading platform for 24 and 48 hours. The overmature packs were sorted from the same lot of fruit and were typical of the berries normally discarded on the plant sorting belt.

All berries used were from one uniform batch, with the exceptions noted (Table 2). The results indicate a relatively high ascorbic acid value for the berries packed in sirup after four months of frozen storage. The overmature and delayed packs contained about 30 per cent less ascorbic acid; relatively more of their ascorbic acid leached into the liquid. The drained berries showed a decrease of over 40 per cent in acidity. The changes in acidity and ascorbic acid were thus much the same for the delayed and the overmature berries. The decrease in flavor and ascorbic acid makes both delay in harvest and in handling undesirable. The further loss on extended storage up to 28 months at —23°C. was not over 25 per cent.

The effects of extended storage, slow defrosting, and holding in the defrosted state at 2°C.(35°F.) are shown (Table 3). These samples were prepared in Gresham, Oregon, in the same way as those in Table 2, and their retention of ascorbic acid during extended storage was equally good. One group of samples was defrosted at room temperature. After sampling, the defrosted packages were placed in a cold room at 2°C. and tested at intervals thereafter. The loss during 24 hours (about 15 per cent) was greater than in samples defrosted entirely at 2°C. for the same length of time.

TABLE 2

Effect of Delay, Overmaturity, and Extended Storage on Ascorbic Acid Content of Frozen Raspberries [1]

Sirup concentration	Frozen storage, 4 months: Drained weight	pH: Liquid	pH: Berries	Citric acid: Liquid	Citric acid: Berries	Ascorbic acid (mg./100 gm.): Liquid	Ascorbic acid (mg./100 gm.): Berries	Ascorbic acid (mg./100 gm.): In blend	Ascorbic acid (mg./100 gm.): Corrected for added sirup	Frozen storage, 28 months: Drained weight	Ascorbic acid (mg./100 gm.): Liquid	Ascorbic acid (mg./100 gm.): Berries	Ascorbic acid (mg./100 gm.): In blend	Ascorbic acid (mg./100 gm.): Corrected for added sirup	Loss between 4th and 28th months
Packed in sirup															
pct.	*pct.*			*pct.*	*pct.*					*pct.*					*mg./ 100 gm.*
50	56	3.24	3.10	0.72	1.49	9.3	16.8	13.5	18.0	54	8.3	11.5	10.0	13.3	3.5
65	57	3.23	3.11	0.71	1.43	10.3	15.0	13.0	17.3	54	8.8	12.6	10.9	14.5	2.1
65	60	3.28	3.13	0.67	1.21	6.6	12.5	11.2	15.0	56	7.5	11.1	9.5	12.7	1.7
Without sirup															
None	63	3.09	3.04	1.41	1.49	14.2	14.4	14.3	14.3	63	12.2	11.4	11.8	11.8	2.5
Delayed 24 hours before freezing															
50	57	3.37	3.25	0.75	1.04	16.6	11.5	11.2	14.9	....	....	....	....	....	....
Delayed 48 hours before freezing															
50	57	3.42	3.37	0.63	0.87	8.4	10.1	9.4	12.5	....	....	....	....	....	....
Overmature—no delay															
50	52	3.42	3.37	0.57	0.78	8.2	10.1	9.2	12.3	....	....	....	....	....	....

[1] The third and fourth lines of data represent samples that were not packed at the same time as the others and hence represent different lots of fruit.

Representative samples frozen without sugar (not shown in the table) were tested without prior defrosting, and then tested after 24 hours at 2°C. The average difference for several samples tested was only 0.7 mg., or about six per cent; the loss in ascorbic acid was thus very small even with no sugar present.

Ascorbic acid is leached from the berries into the sirup during the defrosting process (Table 3). The lots which were defrosted rapidly had more ascorbic acid in the berries than in the sirup; those defrosted slowly had more ascorbic acid in the juice than in the berries at the time of testing.

The loss was greater during extended defrosting in samples without added sugar. Sugar appears to reduce losses of ascorbic acid during defrosting, as it does with strawberries, Loeffler (1946). The loss of 50 per cent or more of the ascorbic acid during four to seven days' holding at 2°C. was not accompanied by the development of any "off" or oxidized flavor.

TABLE 3

Effect of Frozen Storage for 28 Months and Slow Defrosting on Ascorbic Acid of Raspberries

Sirup concentration	Drained weight	Soluble solids		Ascorbic acid (mg. per 100 gm.)						
				After defrosting at room temperature			After holding at 2°C. (35°F.)			Loss
		Liquid	Berries	Liquid	Berries	Blend	24 hr.	48 hr.	96 hr.	
pct.	*pct.*	*pct.*	*pct.*							
50	54	24.5	14.4	8.3	11.5	10.0	8.3	8.2	4.7	5.3
65	54	32.2	17.6	8.8	12.6	10.9	9.5	8.9	5.5	5.4
Dry sugar	54	38.0	23.2	9.2	11.2	10.3	8.9	7.3	....	....
65	56	32.7	15.5	7.5	11.1	9.5	8.0	6.3	3.0	6.5

Sirup concentration	Drained weight	Soluble solids		Ascorbic acid (mg. per 100 gm.) after various periods of holding at 35° F.						
				24 hours			48 hr.	72 hr.	1 wk.	Loss
		Liquid	Berries	Liquid	Berries	Blend	Blend	Blend	Blend	
pct.	*pct.*	*pct.*	*pct.*							
50	58	25.5	14.6	11.3	9.5	10.2	9.2	9.0	6.1	4.1
50	53	23.9	16.5	11.1	10.4	10.7	9.4	7.7	5.5	5.2
Dry	63	9.1	9.7	12.3	11.4	11.8	8.3	8.5	5.4	6.4
50	57	24.2	18.5	10.5	9.6	10.0	10.0	9.2	5.7	4.3

The data on soluble-solids content (Table 3) indicate that 65-per cent sugar sirup does not introduce significantly more sugar into the berries themselves than does the 50-per cent sirup. The stronger sirup merely increases the sweetness of the "cut-out" sirup.

The berries with dry sugar added had about the same retention of ascorbic acid during the extended, frozen-storage period but slightly less retention during 48 hours' holding at 2°C. This was the only case in which sugar content of the berries was increased significantly over that found with 50-per cent sirup, and that increase may have been due partly to adhering sugar. A considerable amount of undissolved sugar was in the packages at the time of defrosting but was mixed in and dissolved before the drained-weight and liquid-soluble-solids readings were taken.

EFFECT OF PURÉEING

Raspberries of the Ranaree variety grown in northern California were used in tests on purées. After one pressing in a tapered-screw pulper, no significant loss of ascorbic acid could be detected. Both the berries and the purée had 26.5 mg. of ascorbic acid per 100 grams; the fibrous refuse and seeds contained 19 mg. per 100 grams. A similar lot of berries was puréed by several passages through a smaller pulper in an attempt to obtain the highest possible recovery of purée. Under this more severe treatment the ascorbic acid decreased from 26.4 mg. to 22.2 mg. per 100 grams of purée. Sugar was added in a 3+1 ratio to this purée, which was tested without prior defrosting after 16 months of storage at —23°C., in one-pound cardboard boxes with water-vapor-resistant cellophane liners. When correction was made for the added sugar the purée contained 19.8 mg. of ascorbic acid per 100 grams. The loss per 100 grams was only 2.4 mg., or about 10 per cent. The contents of the packages were tested after being defrosted by standing 24 hours at 2°C. in the packages. After correction for added sugar, the ascorbic acid content was 18.1 mg. per 100 grams; the loss during defrosting was only 1.0 mg., or about five per cent. The total loss during freezing, 16 months of storage, and 24 hours of defrosting was 3.4 mg., or 15 per cent.

A portion of this same purée was flash-heated before freezing. The flash heater consisted of a rotary, positive-action pump and a straight, ⅝-inch, stainless-steel tube, one half of which was covered with a steam jacket and the other half with a cold-water jacket. Both heating and cooling medium traveled in directions counter to the flow of purée. The purée had been held overnight at 2°C. and contained only 19.8 mg. of ascorbic acid per 100 grams. After heating to a temperature of 82 to 85°C. (180 to 185°F.) for 15 seconds and cooling to below 49°C.(120°F.) in the same interval, the ascorbic acid content was 19.5 mg. per 100 grams.

A portion of this flash-heated material, without added sugar, was tested without defrosting after 16 months of frozen storage at —23°C. in one-pound, waxed cardboard boxes and water-vapor-resistant cellophane liners. No loss of ascorbic acid had occurred during this storage. When the small packages were tested after 24 hours of defrosting at 2°C. the ascorbic acid had dropped to 18.5 mg. per 100 grams, a decrease of only one mg., or about five per cent.

The effects of different types of pulping on ascorbic acid are shown (Table 4). The first three lots of fruit were puréed with a small pulper equipped with conical screen and tapered screw. Since this pulper was small, the fruit was passed through several times. This repetition involved more severe maceration, air incorporation, and delay than would occur in a commercial operation. Despite the nature of this pulping the loss averaged only 1.5 mg. per 100 grams, or seven per cent. Part of the fruit was put through a large-capacity, centrifugal, hammer-mill-type of disintegrator, where a large amount of air was introduced. This very severe treatment destroyed 3.4 mg. per 100 grams, or 16 per cent, of the ascorbic acid. The effect was probably due to the close contact with oxygen rather than to violence of the maceration and disintegration. Disintegration was

also accomplished in a relatively inert atmosphere by the use of liquid seals on the discharge outlets for both purée and refuse and a positive pressure of carbon dioxide in the chamber to sweep out most of the oxygen coming in with the fruit. Under such conditions the loss of ascorbic acid was reduced to 1.1 mg., or only five per cent. The disintegrator in this experiment was the first step of a continuous process involving a Pulley type of deaerator, Pulley (1936), and a slush freezer. The five-per cent loss was measured after the purée was slush-frozen and thus includes not only the losses during the disintegration but also that during the deaeration and slush-freezing stages as well.

TABLE 4

Retention of Ascorbic Acid During Puréeing of Raspberries

Types of pulping	Ascorbic acid			
	Whole	Purée	Loss	
	mg/ 100 gm.	*mg/ 100 gm.*	*mg.*	*pct.*
Several pressings	20.0	19.3	0.7	4
	23.7	21.5	2.2	9
	21.7	20.1	1.6	8
Disintegration	21.7	18.3	3.4	16
Disintegration (inert gas)[1]	21.7	20.6	1.1	5

[1] Disintegration under carbon dioxide was followed by deaeration and slush-freezing. Testing followed slush-freezing.

Commercial purée is handled in packages of 10 to 30 pounds; ascorbic-acid retention under such conditions is shown (Table 5). The samples were frozen, stored, and defrosted in gallon glass jars with air in the head space. These samples, defrosted in 24 hours at 2°C., had somewhat more ascorbic acid than the purée in the one-pound containers when defrosted in the same way.

TABLE 5

Retention of Ascorbic Acid in Raspberry Purée During Freezing, Frozen Storage for 16 Months in Gallon Jars, Defrosting, and Manufacture of Velva Fruit

Ascorbic acid (mg./100 gm.)				
Before freezing	After 16 months of frozen storage and defrosting 24 hr. at 2°C. (35°F.)		After manufacture into Velva Fruit	
	In blend	Corrected for added sugar	In product	Corrected for added ingredients
Unsugared				
26.5	19.1	19.1	12.1	18.6
3+1 sugar				
22.2	14.5	19.3	12.8	18.9
Flash-pasteurized				
19.5	18.4	18.4	11.9	18.4

Sugar had a definitely protective effect on the ascorbic acid of this purée. The unsugared purée lost 7.4 mg., or 28 per cent, while the sugared purée lost only 2.9 mg., or 13 per cent. Flash-heating had an even greater protective effect; heated, unsugared purée lost only 1.1 mg., or 5.6 per cent.

EFFECT OF MANUFACTURE INTO VELVA FRUIT ON RETENTION OF ASCORBIC ACID

The retention of ascorbic acid in raspberry purée manufactured into Velva Fruit is given (Table 5). Velva Fruit is a new frozen fruit dessert developed at the Western Regional Research Laboratory and is composed of frozen-fruit purée, sugar, and an added stabilizer, such as gelatin, Loeffler (1944). The product is processed in an ice cream freezer and, as in ice cream, an equal volume of air (100 per cent overrun) is incorporated. Despite this intimate contact with oxygen, the results show (Table 5) that the loss averaged only 0.3 mg. The flash-heated purée did not lose a detectable amount.

DISCUSSION AND SUMMARY

Raspberries retain their ascorbic acid very well during freezing, processing, and storage. Essentially all of their ascorbic acid was retained during freezing and storage at —23°C.(—10°F.) for periods up to four months. The loss was not over 25 per cent when storage was extended to 28 months. On the other hand, raspberries lose their ascorbic acid and titratable acidity if delayed between picking and freezing or allowed to become overmature on the vine. Consumers may thus receive more ascorbic acid and general satisfaction from frozen raspberries consumed just after they have been defrosted than from fresh-market raspberries. Sugar is of definite value in retaining ascorbic acid during freezing and storage, though raspberries retain their ascorbic acid well in its absence.

Sugar has a pronounced effect on retention of ascorbic acid in raspberry purée. Unsugared bulk purée lost over twice as much ascorbic acid as sugared purée during freezing, 16 months of storage, and defrosting. Since sugar is usually required in the utilization of purée, it should be added before freezing rather than before use.

The protective effect of sugar was pronounced when defrosted berries were held in a household refrigerator. With defrosting in about 24 hours at 2°C.(35°F.) the loss was slight, even in the unsugared berries; but when berries were held several days in a refrigerator, over half of the ascorbic acid was lost. The loss then was 25 to 50 per cent greater in the absence of sugar.

The protective effect of sugar was not found to be proportional to the concentrations of sugar used in the sirups. During freezing and storage, retention was equally as good in 40- or 50-per cent sirup as in 68-per cent sirup.

During holding in a refrigerator after defrosting the loss in the 65-per cent sirup was at least as great as that in 50-per cent sirup. Even where soluble-solids content was raised to a high level with added dry sugar the retention during frozen storage or extended defrosting was not improved.

The stronger sugar solutions have certain disadvantages. "Fondant" formations appeared in packs containing 65-per cent sirup but not in those containing 50-per cent, Rabak (1944). With added dry sugar the defrosted packages of berries contained considerable undissolved sugar. Tasters considered the packs with 50-per cent sugar sirup more desirable than those with 65-per cent sirup or dry sugar. From the standpoint of ascorbic

acid protection there is no disadvantage in the use of the weaker sugar concentration.

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RETENTION OF ASCORBIC ACID IN SOLID-FRUIT AND VEGETABLE-JUICE CONCENTRATES[1,2]

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The recent war focused attention on the conservation of metal and glass in the transport of foodstuffs and on reductions in shipping weight and space. Since most food products contain large percentages of water, such economies may be effected by the partial or complete removal of the naturally occurring moisture.

Within recent years fruit and vegetable juices and their blends have gained considerable popularity because of their palatability and nutritive value. Concentrated juices of approximately 65° Brix have been found by hospitals and other large institutions to be economical and convenient to use for dilution to single-strength juices. Even greater reductions in shipping weight and space have been achieved by the production of solid-juice concentrates of candy-like consistency through the almost complete removal of water *in vacuo*. One concentrated product of this type has been designated as "Bruceets" and has been described by Geer (1944) and Bruce (1945). The pleasing flavor and high nutritive value of the candy-like tablets made from orange and carrot juices suggested their immediate use in concentrated army rations and as a confection in the post-war years. Other concentrates of fruit and vegetable juices, while not having a particularly high vitamin content, are pleasing to the palate and their vitamin content can be readily increased through the addition of crystalline vitamin.

A study of the ascorbic acid retention in these concentrates over periods of time and at various temperatures corresponding to possible conditions encountered in storage or transport is of practical importance. This investigation deals with solid concentrates of apple and carrot juices fortified to various initial levels with crystalline ascorbic acid and stored at different temperatures for varying periods of time.

EXPERIMENTAL PROCEDURE

For this study two readily available juices, apple and carrot, were selected. Although both of these juices have a relatively low ascorbic acid content, the concentrates prepared from the apple juice have a pleasing flavor, while those from the carrot juice are rich in carotene.

The procedure used in preparing the solid concentrates consisted of the followings steps: Clean apples were ground in a hammer mill and the juice collected after expression in a hydraulic press. Pasteurization at

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[1] Journal Paper No. 645 of the New York State Agricultural Experiment Station, Cornell University, Geneva, N. Y.

[2] This investigation was made possible through a grant from Prime Products Inc., Tampa, Florida.

70°C.(158°F.) inactivated the naturally occurring enzymes. When the juice had cooled to 45 to 50°C. (113 to 122°F.) 4.5 grams of a commercial pectic enzyme preparation, Pectinol A, and 100 milligrams of a diastase, takadiastase, were added to each gallon. After incubating at this temperature for approximately one hour and straining through cheesecloth, the juice was introduced into a vacuum pan for concentration.

In the preparation of the carrot juice, abrasion peeled carrots (of the Chantenay variety) were comminuted in an R.Y.P. juicer. The pH of the collected juice was adjusted to 4 to 4.5 with citric acid, pasteurized at 70°C., incubated at 45 to 50°C. with Pectinol A and takadiastase, and then strained.

Throughout most of the concentration the temperature was approximately 43°C.(110°F.). When little water remained, the temperature of the residue rose steadily until, in the case of apples, it reached approximately 95°C.(203°F.) and in carrots, 82°C.(180°F.). At these temperatures the concentrates were removed. The crystalline ascorbic acid was added after breaking the vacuum when the concentration was nearly completed and while the residue was still fluid enough to permit adequate mixing with the vitamin.

When the concentration was judged complete, the residue was removed and, while hot, shaped into small cubes. Each of these, weighing about two grams, was wrapped in moisture-proof cellophane and placed over calcium chloride in desiccators for storage at the following temperatures: 1 to 3°C. (33 to 37°F.); room temperature, which averaged 23°C.(73°F.); 37°C. (98°F.); and 45°C.(113°F.). Samples were removed from storage at monthly intervals for vitamin C analysis by the Mack and Tressler (1937) visual titration procedure. The nature of the vitamin C concentrates permitted direct acid solution without trituration with sand and centrifuging. The dye was standardized daily against crystalline ascorbic acid. The procedure of Bessey (1938), for the estimation of dehydroascorbic acid by regeneration with hydrogen sulfide, was used but no evidence of the presence of the vitamin in this form was found.

RESULTS

To determine whether the losses of ascorbic acid during storage were proportional to the initial fortification levels, the samples were prepared with five widely differing amounts of added ascorbic acid. The initial concentration of this vitamin in the solid-apple concentrates varied from 3.00 to 15.56 mg. per gram and in the carrot concentrates from 9.68 to 29.33 mg. per gram.

The losses of ascorbic acid from the solid-apple concentrates fortified to different degrees with vitamin C and stored at four temperatures are illustrated (Fig. 1). To summarize this graph, the data on losses of ascorbic acid have been averaged and expressed as percentages of the original potency (Table 1). From this table and graph it can be seen that there was negligible destruction of the ascorbic acid in those concentrates stored at 0°C.(32°F.) after as long a period as eight months. Increasing the storage temperature to that normally encountered on store shelves, 23°C.(73°F.), brought about only a slight reduction in the ascorbic acid content. Where

the concentrates were exposed to elevated temperatures of 37°C.(98°F.) and 45°C.(113°F.), however, serious losses of ascorbic acid occurred within as short a period as three months.

At all four storage temperatures there was no indication that the samples with the greater addenda of ascorbic acid suffered greater percentage losses than those to which less had been added. Thus the concentrates with the higher ascorbic acid content maintained their superiority throughout the storage period.

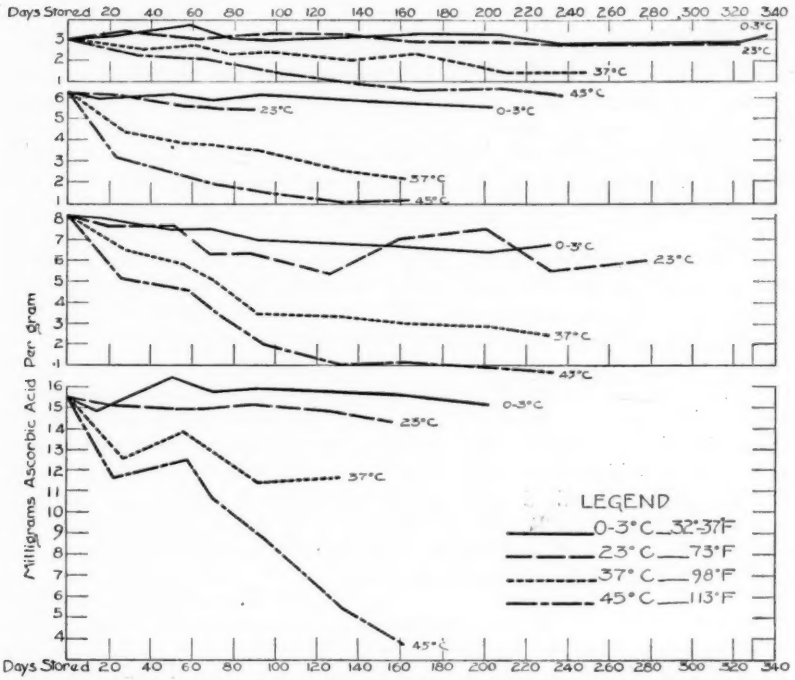

FIG. 1. Losses of ascorbic acid from fortified apple concentrates stored at four temperatures.

The magnitude of the losses of ascorbic acid from the carrot product was essentially the same as that obtained in the case of the apple samples. The results obtained with the carrot concentrates, however, were somewhat irregular. This was probably due to incomplete blending of the ascorbic acid crystals with the semisolid so that a homogeneous mixture was not obtained.

SUMMARY

Solid concentrates of apple and carrot juices were prepared which were palatable and, through fortification, possessed appreciable quantities of ascorbic acid. These concentrates were fortified to different levels of ascorbic acid potency and stored at various temperatures. From the results

TABLE 1

Change in Ascorbic Acid Content of Fortified Apple Concentrates During Storage

Storage temperature	Change in ascorbic acid content (per cent) after days in storage:											
	16	24	29	53	58	72	93	130	163	205	234	281
°C.												
0	+1	....	....	—5	....	—1	—3	....	—4	—6	—9	+3
23	....	+1	....	—3	....	—8	—9	—9	—10	—9	—23	—16
37	....	....	—20	....	—24	—31	—40	—44	—54	—54	—62	....
45	....	—34	....	....	—35	—49	—64	—78	—82	—86	—90	....

of the storage studies it appears most advisable to avoid storage at temperatures in excess of room temperature, or 23°C.(73°F.). When feasible, storage under refrigeration at 0°C.(32°F.) will safeguard the ascorbic acid content for long periods of time. Where high storage temperatures are unavoidable, solid-juice concentrates should be consumed as soon as possible after production. In samples stored under similar conditions, a proportionate destruction of ascorbic acid was noted regardless of the initial level of fortification.

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INVESTIGATION OF *SALMONELLA* CONTENT OF POWDERED WHOLE EGG WITH NOT MORE THAN TWO PER CENT MOISTURE CONTENT

III. EFFECT OF PREHEATER ON SURVIVAL OF SPECIES OF *SALMONELLA* DURING COMMERCIAL SPRAY DRYING

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In a previous report, Schneider (1946), of a survey on egg powder it was shown that the use of the preheater and multistage drying affected the survival of *Salmonella* organisms. The following study is a continuation of the previous report.

Gibbons and Moore (1944) studied the effect of an experimental dryer on the survival of *Salmonella* organisms. The present study concerns the survival of *Salmonella* organisms actually encountered during commercial spray drying of high-quality egg powder. Three plants, presenting some differences in mechanical equipment, participated in the study.

EXPERIMENTAL PROCEDURE

Two studies were made of plant No. 6 which operated with two multistage drying units of the Rogers type. Both units received the raw egg simultaneously from a single source of supply. In each instance, one dryer operated with the aid of the preheater, the other without it.

Plant No. 2 used a single dryer of the Rogers type, a preheater being used on the second of two days.

Plant No. 9 processed egg powder with a Douthitt type spray dryer. In addition to the preheater, a holding (pasteurizing) vat was used which maintained the preheated liquid egg at 60.6-61.7°C.(141-143°F.) for approximately six minutes prior to spray drying. One day of drying was accomplished with, and the following day without, the aid of the preheater and holding vat.

Examinations were made of several egg-pulp samples collected during the course of each day's drying. When preheaters were used, collections of egg pulp were made before and immediately after preheating. Each aliquot of egg powder examined represented an hour's production of approximately 500 to 600 pounds.

Isolation of *Salmonella* organisms, biochemical studies, and serological typing were the same as previously reported by Schneider (1946). Standard plate counts were determined in accordance with the method described in the "Standard Methods for the Examination of Dairy Products," APHA, eighth edition, 1941, with exceptions as specified in the Quarter-

[1] This work was done while the author served as Captain in the Veterinary Corps, U. S. Army, Seventh Service Command Medical Laboratory.

master Tentative Specifications CQD 169A and CQD 117B. Exceptions read as follows:

"Prepare a 1:10 dilution by weighing aseptically and accurately a 5-gram sample of powdered egg into a 120-ml., wide-mouth, glass-stoppered bottle containing 45 ml. of sterile distilled water and a teaspoonful of sterile glass beads. Shake in a Kahn or similar automatic shaker for one minute. In addition, agitate the contents of each bottle thoroughly and vigorously by shaking 25 times, each shake being an up and down excursion of about a foot, before the removal of any portion of any sample or its subsequent dilution. Transfer 11 ml. of the 1:10 dilution into a sterile 99-ml. distilled water blank, contained in a 120-ml., narrow-mouth, glass-stoppered bottle. This results in a 1:100 dilution. With a sterile 1-ml. pipette transfer 1 ml. of the 1:10 dilution into a sterile 99-ml. distilled water blank. This results in a 1:1,000 dilution. With the same pipette inoculate duplicate petri plates with 1 ml. each of the 1:10 dilution and set aside for the yeast and mold determination. With a second sterile 1-ml. pipette transfer 1 ml. of the 1:100 dilution into a sterile 99-ml. distilled water blank. This results in a 1:10,000 dilution. With the same pipette inoculate duplicate petri plates each with 1 ml. of the 1:100 dilution for the Standard Plate Count. With a third sterile 1-ml. pipette inoculate 1 ml. of the 1:10,000 dilution into each of duplicate petri plates. With the same pipette then inoculate 1 ml. of the 1:1,000 dilution into each of duplicate petri plates. Pour the plates with 15 ml. of melted and cooled (45°C.) Standard Methods nutrient agar (old type). Mix well and allow plates to harden. Invert the plates and incubate at 32°C. for 72 hours."

RESULTS

Plant No. 6: On November 15, 1944, this plant showed contamination of the raw egg with *S. oranienburg* during the first hour of operation. This species of *Salmonella* was isolated from the egg powder processed without the aid of the preheater during the third, fourth, and seventh hours of production but was not recovered from the preheated liquid egg or the egg powder processed from the latter. However, *S. tennessee* was isolated from the first hour's production with the preheater; but since the viable count of the preheated liquid egg in the first hour of operation was not significantly reduced from that found in the raw material, it apparently indicated the preheater was not functioning efficiently at that time.

On November 25, 1944, in the first two hours of processing, the raw material was contaminated with *S. pullorum, S. oranienburg,* and *S. tennessee.* Frequent recoveries were made only of the species *S. pullorum* from egg powder processed without the preheater. No *Salmonella* organisms were isolated from the preheated liquid egg or the subsequent dried powder in spite of the fact that there was a definite increase in the plate count of egg powder from the 13th to the 21st hour of operation.

On February 15, 1945, an attempt was made to recover *Salmonella* organisms from preheated liquid egg. While the raw material was contaminated with *S. pullorum* up to the eighth hour, no recoveries were made of *Salmonella* organisms from the preheated liquid egg throughout a 19-hour period.

Plant No. 2: On December 28, 1944, the raw liquid egg yielded *S. oranienburg.* Without the preheater five recoveries of *S. oranienburg* and seven isolations of *S. pullorum* were made from the egg powder. In contrast, operating with the aid of the preheater on December 29, 1944, no isolations of *Salmonella* organisms were made from either the preheated egg or from the egg powder produced from it, although the raw material was contaminated with *S. oranienburg* and *S. pullorum.*

Plant No. 9: On March 1, 1945, processing without the preheater, the raw material as well as the egg powder yielded cultures of *S. pullorum*. On March 2, 1945, during spray drying with the aid of both the preheater and holding (pasteurizing) vat at 141-143°F. for about six minutes, *S. pullorum* was isolated from the raw and the preheated liquid egg. No isolations were made from the liquid egg held in the pasteurizing vat or from the subsequent dried-egg powder.

TABLE 1

Isolation of Species of Salmonella During Commercial Drying

Liquid [1]	Number examined	Number positive	Powder [1]	Number examined	Number positive
Plant No. 6—November 15, 1944					
R	3	1	DoP	20	3
P	3	0	DiP	20	1
Plant No. 6—November 25, 1944					
R	5	3	DoP	21	11
P	4	0	DiP	20	0
Plant No. 6—February 15, 1945					
R	5	2	.....	....	....
P	19	0	.....	....	....
Plant No. 2—December 28 and 29, 1944					
R	10	9	DoP	14	10
P	5	0	DiP	17	0
Plant No. 9—March 1 and 2, 1945					
R	8	3	DoP	12	2
P	14	1	DiP	14	0
H	14	0	.....	....	....
All Plants					
R	31	18 or 59.1%	DoP	67	26 or 38.8%
P	45	1 or 2.2%	DiP	71	1 or 1.4%
H	14	0	.....	....	....

[1] R—liquid egg, raw; H—liquid egg, holding; P—liquid egg, preheated; DoP—dried without preheater; DiP—dried with preheater.

Summarized data of each and of all the plants studied are shown (Table 1). In the latter, a 59.1-per cent contamination of the raw material was found. While 38.8 per cent isolations of *Salmonella* organisms were made from egg powder processed without the preheater, isolations from preheated liquid egg were 2.2 per cent and egg powder produced from the latter, 1.4 per cent. Fourteen samples of liquid egg being held in the pasteurizing vat yielded no *Salmonella* organisms.

DISCUSSION

Gibbons and Moore (1944) demonstrated that over 99 per cent of *Salmonella* organisms in liquid egg were killed on drying in an experimental dryer. It is not known whether preheaters were used in their study. They reasoned from their experiment that comparable results would be obtained in commercial dryers. We are in agreement with this conclusion. However, it is to be noted that a 99-per cent reduction of viable *Salmonella* organisms

does not mean the exclusion of these bacteria from the egg powder. Even with a 99-per cent reduction there may still be significant numbers of organisms remaining viable in the egg powder, depending upon the degree of contamination of the raw material.

It is evident from the results shown (Table 1) that the preheater is unusually effective in the destruction of *Salmonella* organisms. During commercial processing only one isolation, a culture of *S. pullorum,* was made from 45 specimens of preheated liquid egg. In the egg powder produced with the aid of the preheater one isolation, or 1.4 per cent, of *S. tennessee* was made from 71 samples examined. In the latter instance, since this organism had not been isolated from the raw material, it is not known with certainty that it had passed through the preheater. Possible explanations for the isolation of this culture are (1) data in results appear to show that the preheater was not operating efficiently during the first hour of production and hence it may have been present but had not been isolated from the raw material; (2) contamination may have occurred during cleaning of the dryer.

The low percentage, 1.4 per cent, isolation of *Salmonella* organisms compares favorably with a survey incidence of 1.63 per cent in egg powder processed with the aid of the preheater, Schneider (1946). In the absence of the preheater, 38.8 per cent isolations of *Salmonella* organisms were made.

CONCLUSION

While the preheater was primarily intended to lower the moisture content, this study shows that it is also a very effective measure for the exclusion of *Salmonella* organisms from powdered whole egg with not more than two per cent moisture content.

SUMMARY

Commercial dryers of the Rogers and Douthitt types, using preheaters, show a very significant reduction of the *Salmonella* content of whole-egg powder with not more than two per cent moisture content.

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MEASUREMENT OF BEEF TENDERNESS AND TENDERIZATION OF BEEF BY TENDERAY PROCESS [1]

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The consumer desires flavor and tenderness in meat more than any other qualities. Even when meat has good flavor, it is still undesirable if it is tough. Also tender meat must not be "off-flavor" in order to be acceptable to the consumer. Usually fresh meat does not have objectionable flavor characteristics, but for the most part fresh meat is tough, particularly beef. In order to promote tenderness in beef, it may be tenderized or ripened by hanging at refrigerator temperatures of 0.6 to 1.1°C. (33 to 34°F.) for varying lengths of time from three to six weeks. In this ripening process there is sometimes danger of inducing off-flavors in the meat. Further, the trimming and shrinkage losses and storage costs are so high in ripened beef that its increased cost makes it more expensive than fresh beef to the consumer. In order to circumvent these and other disadvantages to beef ripening, the Tenderay process was developed [Researches at Mellon Institute (1940, 1941); James (1939); Knowles and Reuter (1940); Rentschler and Nagy (1940); and McCarthy and King (1942)]. Since these initial studies an extensive research program on beef tenderization and the Tenderay process has been under way in our laboratories. In order to follow the tenderization of meat by this or other processes, it was necessary to devise a more satisfactory test for tenderness than had been available heretofore. The testing procedure described here is the result of considerable effort to get a reliable test. The merits of this technique are discussed in relation to the tenderness studies involving comparison of "Tenderayed" and unprocessed beef.[2]

There are many mechanical testers described in the literature [Black, Warner, and Wilson (1931); Bratzler (1932); Winkler (1939); Steiner (1939)] and some have received a measure of acceptance by investigators in meats. We have not found these and other mechanical tests developed in our laboratory sufficiently reproducible and reliable for the reasons that meat is such a heterogeneous food and that tenderness values so obtained are dependent on many factors difficult to control, such as temperature of the sample to be tested, sample size, position of the sample in the mechanical tester, and elapsed time between preparation and testing. Furthermore, we have found that mechanical testing, as done in our laboratories, saves no time compared with organoleptic panel testing, particularly when a large number of samples have to be run. It is true that mechanical testing dispenses with the subjective human element to

[1] Included in the 1945 Meeting-in-Print of the American Chemical Society.

[2] The term "unprocessed beef" refers to beef that has not been aged or ripened, "Tenderayed," or put through any other tenderizing procedure.

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some extent but results so obtained are still empirical and arbitrary in interpretation. Indeed the basic reference for any mechanical tester is still taste-testing for tenderness; and when this is not done, tenderness data are sometimes quite misleading. We are not unmindful of the merits of mechanical tenderness testers and have not ceased our search for such a device, but as yet we have found none which we think correlates satisfactorily with taste-testing.

In our beef-tenderness investigations to date we have relied on panel taste-testing for the reasons given above and also since such testing is more analogous to the measure of consumer acceptance. The taste-testing technique of the Co-operative Meat Investigations Committee has been used to evaluate meat tenderness by many investigators with apparent satisfactory results. The paired testing of Cover (1936, 1940) is somewhat more adaptable to tenderness studies.

For our investigations on the tenderization of meat a somewhat different tenderness-testing technique has been devised through the co-operative efforts of our entire organization and is not the particular work of the authors themselves.

EXPERIMENTAL PROCEDURE

The test is particularly applicable to tenderness studies in that it is comparative and at the same time allows tenderness evaluation for each sample tested. Our beef-tenderness studies are carried out using beef directly from the killing floor of the packing house. The dressed-out carcass is split in the usual manner and each side is treated differently, according to the particular tenderizing process under study. For example, one side of the carcass may be run through the Tenderay process while the matched side may not be processed. Following the completion of the process under investigation, each side is cut down and comparable test samples are taken. Any test sample may be taken, but usually tests are run on matched, broiled short-loin steaks. If roasts are desired, matched rib roasts are taken from the forequarter adjacent to the short loins used for the steaks. In this way suitable test samples may be taken, using a minimum of meat and avoiding mutilation of the remainder of the carcass.

The tasting panel consists of six tasters with some experience. For most tests matched, broiled short-loin steaks one inch thick are used. Although electric broilers may be used, we have found that gas-fired broilers operating at 204.4°C.(400°F.) are better suited to our particular testing. For each test two steaks are taken from the same relative position on each side of the animal (making four steaks in all) and broiled simultaneously to the medium-rare stage.

When done, three one-inch segments are cut from the middle portion of each steak. Coded, matched segments are placed on plates for the judges to grade as to tenderness level and preference. If rib roasts are tested, paired roasts of approximately equal weight are roasted in a 162.8°C.(325°F.) oven to an internal temperature of 71°C.(160°F.) and taste-tested by the six-judge panel. Complete slices from the same relative position of the rib roasts are compared and judged in the same manner as the steaks. For tenderness grading, a 0 to 10 scoring system is used according to the schedule of 10, very tender; 8, tender; 6, slightly tough; 4,

tough; and 2, very tough. Judges are also permitted to score in appropriate odd numbers as they see fit. For preference scores the judges merely designate their choice if they find one sample is more tender than the other.

Most of our testing is done on broiled short-loin steaks for several reasons. The time required for cooking and taste-testing is less than for other cuts. With two broilers capable of holding four steaks each, six tests per hour can be completed. This shorter time, together with the fact that less meat is required when steaks are tested, permits each experimental animal to be tested in duplicate or triplicate by all six judges of the tasting panel. This factor is of utmost importance. Broiled steaks are not as tender as roasts from the same animal and, therefore, differences tend to be greater in steaks. Cooking time is more uniform and the use of steaks permits simultaneous cooking of the samples to be compared.

We have found that testing of meat by comparison of two paired samples which differ only in tenderization process is very desirable as it tends to eliminate errors resulting from biological variation in animals and inherent variation in carrying out experiments on tenderization.

There is some objection to comparative testing on the grounds that when two samples are presented to the judges, they tend to imagine differences which may not exist. Analysis of test data tends to support that supposition but only to a limited degree. Deviations in results owing to such tendency are overshadowed by the advantages of comparative testing particularly when the limitations of the test data are not forgotten when results are interpreted.

In the discussion which follows the tenderness scale of 0 to 10, as noted above, is used. Deviations are given on the single taster basis rather than on the panel basis. A *test* denotes a complete comparative test of two sides of meat by the entire panel, while the term *half-test* indicates only one half of the comparative test and, therefore, represents only one side of meat. Tenderness is a relative organoleptic quality and percentage increase in tenderness is meaningless as an index of improvement. Therefore, increases in tenderness are noted only as units on the tenderness scale. Since we have found no standard reference for tenderness, the mean of duplicate or triplicate half-tests is used as a basis for calculating deviations. Estimated standard deviations were computed using the method of least squares.[3] The probable deviation is considered to be 0.67 of the estimated standard deviation. All deviations are on the single-judge basis rather than on the panel (six-judge) basis.

RESULTS AND DISCUSSION

Reproducibility of Results as a Measure of Reliability: A criterion for the validity of a test is its reproducibility. Since our tenderness studies were followed by duplicate or triplicate testing, estimated standard devia-

[3] Estimated standard deviation $= \sqrt{\frac{\Sigma(T-\overline{T})^2}{m(n-1)}}$

where T = observed tenderness; $\overline{T}$ = mean tenderness; n = number of observations per side; and m = number of sides.

tions from the means of such tests were computed in order to evaluate the reproducibility of the results obtained by our testing technique.

From the accumulated data of our investigations we have analyzed the results of 438 duplicate half-tests representing unprocessed and "Tenderayed" beef including all U. S. grades as received at a packing plant, predominantly U. S. Good and U. S. Commercial with about 10 per cent U. S. Choice and less than one per cent U. S. Utility. When all these data were combined and the estimated standard and probable deviations (from their respective means of the duplicate half-tests) were computed, we found that for a single half-test on the single-judge basis the estimated standard deviation was 0.73 and the probable deviation 0.49.

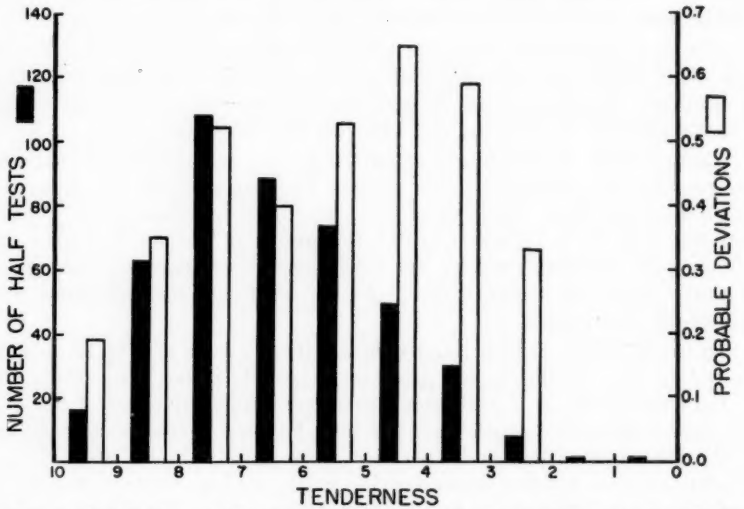

FIG. 1. Distribution of 438 duplicate half-tests over the tenderness scale, and probable deviations of the single half-test in relation to tenderness.

The question arises as to whether these deviations vary with respect to the tenderness level of the meat being tested. Since an arbitrarily bounded tenderness scale was used, diminished deviations as tenderness approaches 10 to 0 were to be expected. We did get these together with maxima in the deviation at tenderness 4 to 5 and at tenderness 7 to 8. We do not offer an explanation for these unexpected maxima in deviations since at the present time that is still in the realm of speculation. Data on these 438 duplicate half-tests are given (Table 1) and the distribution of these tests over the tenderness scale, together with estimated standard and probable deviations at various tenderness levels, are shown (Fig. 1).

In the proper evaluation of results obtained by this tenderness-testing technique it was necessary to know if deviations increased when compared samples approximate each other in tenderness. There is this tendency but it is not of sufficient magnitude to interfere with the usefulness of this testing method. Of the 438 duplicate half-tests (Table 1 and Fig. 1) 286

have been done in one series of investigations in which comparisons of processed and unprocessed beef predominated. The remaining 152 comprise a different series of tests comparing various tenderizing procedures. Consequently, in the first group of tests tenderness differences were greater than in the second group. For the first group the estimated standard and probable deviations for single half-tests were 0.67 and 0.44, respectively, while in the second group these values were 0.84 and 0.56, respectively. Similar computations involving grouping different from the above showed the same tendency.

TABLE 1

Composite Data on 438 Duplicate Half-Tests Showing Distribution Over Tenderness Scale and Variation of Estimated Standard and Probable Deviations With Respect to Tenderness Level

Tenderness level	Number of half-tests	Average tenderness	Estimated standard deviation	Probable deviation
10.00-9.00	16	9.36	0.28	0.19
8.99-8.00	63	8.44	0.53	0.35
7.99-7.00	108	7.47	0.78	0.52
6.99-6.00	88	6.47	0.60	0.40
5.99-5.00	74	5.50	0.80	0.53
4.99-4.00	49	4.57	0.98	0.65
3.99-3.00	30	3.55	0.88	0.59
2.99-2.00	8	2.49	0.50	0.33
1.99-1.00	1	1.92	.....	.....
0.99-0.00	1	0.67	.....	.....
Total half-tests....	438	.....	.....	.....
Deviations representing all tests.....			0.73	0.49

Relation of Preference Scores to Observed Tenderness Differences: If we use the foregoing values of estimated standard and probable deviations as an index of significance in results, we can make an evaluation of significance in preference votes by the tasting panel. Three hundred and five single tests were analyzed comparing the preference votes and the corresponding difference in tenderness scores. For each category of preference votes the mean difference in tenderness was computed on the single-test, single-judge basis (Table 2).

TABLE 2

Comparison of Preference Scores With Observed Differences in Tenderness

Preference vote [1]	Mean difference in tenderness scores
6 -0	2.60
5½- ½	1.31
5 -1	1.24
4½-1½	0.79
4 -2	0.54
3½-2½	0.29
3 -3	0.24

[1] Tie scores are counted ½ for each sample in the test.

These data indicate that for a single test by a six-judge panel preference votes lower than 5 to 1 have questionable or no significance. Of course, on repeated testing the lower preference ratings gain significance on the premise that the error or deviation of the mean of a number of determinations decreases as the square root of the number of determinations increases.

Variation in Individual Judges: Using the deviations of individual judges from the means of respective duplicate and triplicate tests we may get some idea of the estimated standard and probable deviations by the individuals who make up the tasting panel. An analysis of approximately 500 individual tenderness scores representing seven judges shows that the estimated standard deviation is about 1.27 and the probable deviation is about 0.87. This indicates that a judge is usually within one point of the mean tenderness score derived from duplicate or triplicate taste-testing by six individuals.

As might be expected, individual judges vary somewhat in their ideas of tenderness—some are consistently high scorers and others are consistently low. However, estimated standard and probable deviations of individual judges do not indicate much variation in the judges' ability to evaluate tenderness.

When tests were run on the same meat by experienced panels and several inexperienced panels (taken from office and laboratory staffs) the results by the inexperienced judges concurred with those by the experienced judges; however, there was a definite tendency for the inexperienced judges to score meat higher in tenderness.

Comparison of Broiled Steaks and Rib Roasts for Test Purposes: It is well known that meat varies in tenderness with the method and degree of cooking. The work of Cover (1937, 1941) is of interest in this respect. We have observed that steaks broiled to the well-done stage tend to be less tender than steaks broiled to the medium-rare stage. Since most tenderness studies reported in the literature have used rib roasts for test purposes, we have felt it desirable to compare the tenderness of broiled steaks and rib roasts from the same test sides.

In 27 triplicate tests broiled steaks were compared for tenderness and at the same time rib roasts were compared in a similar manner for these 27 test animals. These data, consisting of 54 half-tests, permit an estimation of tenderness differences in cooking by the two methods and offer an excellent opportunity to evaluate the merits of tests using broiled short-loin steaks compared with tests using rib roasts (Table 3).

These data indicate clearly that there is a very close correlation between the tenderness of rib roasts and broiled short-loin steaks; that, in general, roasting produces somewhat more tender meat than broiling; and that the greatest increases in tenderness owing to roasting tend to occur in the tougher meat. From these findings we can say that for tenderness studies broiled steaks have some points of preference as test samples over rib roasts because the tenderizing effort of roasting is eliminated and because more tests can be done in less time, thus permitting more animals to be tested and also a greater number of tests per animal.

Tenderization of Meat by Tenderay Process: During recent investigations we have accumulated data on 82 test animals, one side of each being put through the Tenderay process and the other side representing the unprocessed control. Forty-three of these animals were graded U. S. Good and 39 U. S. Commercial. All of these cattle were taken from the regular production of a packing plant. Each animal was tested in the usual way, and tenderness values of each side were taken as the mean values obtained on duplicate testing of broiled short-loin steaks by the six-taster panel.

TABLE 3

Comparison of Tenderness of Broiled Short-Loin Steaks With Tenderness of Rib Roasts

Number of half-tests	Tenderness level of steaks	Mean increase in tenderness owing to roasting
6	9.00-8.00	—0.12 [1]
23	7.99-7.00	0.62
11	6.99-6.00	0.83
11	5.99-4.00	1.58
3	<4.00	0.73 [1]

[1] Little or no significance is indicated because of insufficient number of comparisons in this tenderness range.

Total number of comparisons	54
Mean tenderness of broiled steaks	6.53
Mean tenderness of rib roasts	7.30
Median tenderness of broiled steaks	7.06
Median tenderness of rib roasts	7.50
Coefficient of correlation	0.83

TABLE 4

Summary of Data Comparing Tenderness of "Tenderayed" and Unprocessed Beef With Respect to Grade

Degree of tenderness	U. S. Good		U. S. Commercial	
	"Tenderayed"	Unprocessed	"Tenderayed"	Unprocessed
Number of tests	43	43	39	39
Mean tenderness	6.50	4.58	6.98	4.46
Median tenderness	6.58	4.58	7.25	4.33
Maximum tenderness	9.50	7.17	8.85	7.75
Minimum tenderness	3.33	2.17	3.50	1.92

The results obtained were analyzed with respect to grade and it was found that the tenderness levels of the "Tenderayed" and unprocessed sides of the U. S. Commercial cattle were comparable to the tenderness of respective sides of the U. S. Good cattle. In fact the U. S. Commercial "Tenderayed" sides were somewhat more tender than the U. S. Good "Tenderayed" sides. For the unprocessed meat the U. S. Good sides were only slightly more tender than the U. S. Commercial; the data are summarized (Table 4).

Whether median tenderness or mean tenderness is used as a criterion of improvement, the U. S. Commercial carcasses improved in tenderness somewhat more than the U. S. Good carcasses as a result of the Tenderay process.

When the above data were combined and analyzed with respect to the distribution over the tenderness scale a further breakdown of data was the result (Table 5 and Fig. 2).

TABLE 5

Composite Data Comparing Tenderness of "Tenderayed" Beef With Unprocessed Beef and Distribution of Tenderness Scores

Tenderness level	Number of half-tests	
	"Tenderayed"	Unprocessed
10.00-9.00	1	....
8.99-8.00	10	....
7.99-7.00	32	4
6.99-6.00	17	5
5.99-5.00	14	22
4.99-4.00	5	22
3.99-3.00	3	19
2.99-2.00	....	9
1.99-1.00	....	1
Total	82	82
Mean tenderness	6.73	4.54
Median tenderness	7.04	4.54

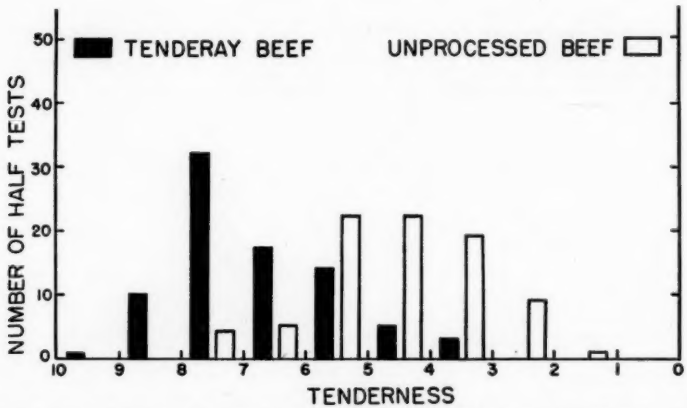

FIG. 2. Distribution with respect to tenderness of half-tests representing the comparison of "Tenderayed" beef and unprocessed beef from 82 test animals coming to slaughter through usual commercial channels.

Inspection of these data and the graphs (Fig. 2) discloses that the tenderness values of the unprocessed beef follow a normal curve while the distribution of tenderness values of "Tenderayed" beef show a skewed curve owing to the limiting nature of the tenderness scale. Under these circumstances the median may be the better criterion for tenderness comparisons and this indicates a 2.50 improvement in tenderness owing to the Tenderay process.

In a group of 16 U. S. Choice cattle, purchased from the Ohio Agricultural Experiment Station following feeding studies, definite improvement

in tenderness owing to the Tenderay process was also found. These cattle were very well finished and far above regular market cattle in this respect. For each of these animals one side was "Tenderayed" and the other side was not; all sides were tested in duplicate using broiled short-loin steaks. The average tenderness was 7.56 for the unprocessed sides and 8.91 for the "Tenderayed" sides. The median tenderness levels were 8.12 and 9.21, respectively. Although the unprocessed sides were graded "tender," there was still noticeable improvement in tenderness owing to the Tenderay process.

Further study of the improvement in tenderness owing to the Tenderay process indicated that different animals show variation in magnitude of improvement in tenderness. Some meat shows great improvement while other meat shows only noticeable improvement. The tenderness increases may range from a fraction of a point to as much as six points on the tenderness scale. Usually most carcasses increase two to three points.

SUMMARY

A method for the organoleptic evaluation of tenderness in meat has been described, and the method is applicable to broiled short-loin steaks. The method also permits comparison of two meats for differences in tenderness and provides a method for preference scoring as well as tenderness scoring by the judges.

The results obtained in 438 duplicate half-tests permitted computation of estimated standard and probable deviations as indices to reproducibility of results. The probable and estimated standard deviations for the single half-test and for individual judges were determined. Preference scores and differences in meat tenderness scores were compared. The evaluation of these characteristics of test data facilitate better planning of experiments and interpretation of results.

The tenderness of broiled short-loin steaks and the tenderness of adjacent rib roasts from 54 sides of beef were compared. The results indicate a tenderizing action owing to roasting, particularly in the less tender sides. Correlation of results indicate that broiled short-loin steaks are as good as rib roasts for testing and have some points of superiority for that purpose.

The tenderizing effect of the Tenderay process was determined on 82 animals coming to slaughter through regular channels. The tenderness of the U. S. Commercial animals was comparable to the tenderness of the U. S. Good animals, both unprocessed and "Tenderayed." The U. S. Commercial carcasses showed somewhat greater improvement than the U. S. Good. For the whole group the "Tenderayed" meat scored 2.5 points higher in tenderness than the unprocessed meat, using median tenderness as a criterion for comparison. The median tenderness for the unprocessed beef was 4.54 and for the "Tenderayed" beef, 7.04.

In a single lot of 16 U. S. Choice cattle purchased from the Ohio Agricultural Experiment Station the median tenderness of the unprocessed sides was 8.12 and for the "Tenderayed" sides it was 9.21, indicating a noticeable tenderization on beef which was classified as tender without processing.

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ASCORBIC ACID CONTENT OF PINEAPPLES AS CORRELATED WITH ENVIRONMENTAL FACTORS AND PLANT COMPOSITION

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The ascorbic acid content of plants may vary widely depending upon the conditions under which the plants are grown, Maynard and Beeson (1944). In recent work with tomatoes relatively great variations in ascorbic acid were found correlated with the season and location at which the plants were grown, while little influence of the supply to the plant of macronutrient and micronutrient elements was apparent [Hamner, Lyon, and Hamner (1942) and Lyon, Beeson, and Ellis (1943)]. The work of many investigators [Hamner and Parks (1944); Reder, Ascham and Eheart (1943); Reid (1941, 1942); and Murphy (1942)] with numerous plants has indicated that such climatic environmental factors as air temperature, light intensity, length of day, and rainfall have an influence on ascorbic acid values. On the other hand, there is much disagreement among investigators as to whether or not soil conditions and fertilizer practices exert any effect.

A study of the ascorbic acid content of pineapple fruit in Hawaii was thought to be profitable, especially since variations in the vitamin could be followed in conjunction with variations in climate and plant composition. Only one variety of pineapple is grown and all plants are vegetatively propagated so that genetic differences between plants are not of concern. Climatic differences from one location to another a few miles away are relatively great. Plants are grown under conditions of temperature that differ materially at elevations approximating sea level to others over 2,000 feet. Light intensities vary from 2,000 to 8,000 foot candles, depending upon season and location. Differences in rainfall may be very great. Fairly complete records of these climatic variables are kept for each field location. In addition, careful records of fertilizer practices are available. Studies on the chemical composition of the plants and the fruit are extensive. With all of these data available it was thought that variations in ascorbic acid might be correlated with some particular variable of climate or fertilizer practice, or that a correlation between the vitamin content and some other plant constituent might become apparent.

EXPERIMENTAL PROCEDURE

The ripe fruits from the experimental plots were harvested and processed to form juice in a commercial cannery. The samples of canned juice were shipped to the United States Nutrition Laboratory at Ithaca, New York, and stored at 5.6°C.(42°F.). The vitamin analyses were conducted as soon as possible thereafter.

[1] Hawaiian Pineapple Company, Honolulu, Hawaii.

Individual samples of juice from several cans were analyzed for ascorbic acid by the method of Bessey (1938). Certain samples of juice were analyzed immediately upon arrival at the Laboratory, and other cans from the same lot were analyzed after several months' storage in the refrigerator. No evidence of a decrease in the ascorbic acid content was noted. A number of the various samples was analyzed for dehydroascorbic acid; the amount of this substance was low in all samples and the amount in the various samples bore approximately the same quantitative relationship to the amount of ascorbic acid. Therefore, none of the values for dehydroascorbic acid are presented in this paper.

A general discussion of the cultural practices followed in the production of pineapples in Hawaii is given in a previous paper by Nightingale (1942). In that article details are given of the procedures followed in making observations in the field, obtaining samples for chemical analysis, recording variations in the environmental factors; and other pertinent data are included. A discussion is also given of the effect of various fertilizer treatments on growth, fruitfulness, and chemical composition. In the present experiments, the same procedures were followed with respect to the cultural practices, the recording of data, and the selection of plants as already described by Nightingale (1942) to represent a given plot.

Experiment I: Conducted in Field 4311, this experiment was designed to study the effects of nitrogen fertilization. Plants in Plots A received 272 lb. per acre of nitrogen, while those in Plots B received 432 lb. At the time of floral differentiation there were marked differences between the two lots of plants. Those of B had relatively large reserves of nitrate in relation to No. 1 leaf color.[2] The B plots produced two and one-half tons more fruit per acre than the A plots. The appearance of the plants, chemical composition, and yield indicated that from a practical standpoint the two treatments, consisting of six replicates of each, represented fairly extreme conditions of nitrogen nutrition.

Experiment II: This experiment was also conducted in Field 4311. These plants all received N—430 lb., K_2O—300 lb., P_2O_5—305 lb. per acre. The plants of both groups were somewhat deficient in nitrate in relation to carbohydrates, and from the standpoint of yields were inadequately supplied with potassium and low in phosphorus at the time of floral differentiation. Plants in Plots A received no additional fertilization, while those in Plots B were supplied with sulfate salts of Zn, Mn, Cu, and borax at the following rates each month as a spray: Zn—9.5 gm., Mn—9.4 gm., Cu—4.8 gm., B—1.4 gm. No response to the micro-element fertilization was noted, and the plants of the two groups were essentially similar in appearance and in determined constituents.

Experiment III: This experiment was conducted in Field 4435. There was little temperature difference between the two fields just previous to harvest. Plants in Plots A received 272 lb. of nitrogen per acre and those of B, 432 lb. Both plots received potassium at the rate of 150 lb. of K_2O per acre, and neither group received phosphorus fertilizer. However, both

[2] No. 1 leaf color, described by Nightingale (1942), is intimately correlated with carbohydrate reserves, the higher the No. 1 color the higher the carbohydrate content.

TABLE 1

Ascorbic Acid Content (mg. pct.) of Pineapple-Juice Samples (Arranged in Ascending Order of Ascorbic Acid Value)

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Expt.	Field	Harvest date	Day length[1]	Light rate		Air temp.		Fruit temp.	At floral differentiation					At time of harvest		Fruit					
				Weeks[2]		Weeks[2]		Weeks[2]	Leaf values												
				6	2	6	2	2	No. 1 color	Nitrate N	K	P	Plant wt.	No. 1 color	Plant wt.	Exposure	Weight	pH	Brix	Acid as citric	Ascorbic acid
			hr.	*f.c.*	*f.c.*	°*F.*	°*F.*	°*F.*	*pct.*	*pct.*	*pct.*	*pct.*	*lb.*	*pct.*	*lb.*	*pct.*	*lb.*			*pct.*	
1A	4311	7-23-41	13:09	5200	5300	75.2	75.5	77.1	32	.041	.....	.....	9.7	13	9.7	58	4.4	.....	16.1	0.47	2.9
2A	4311	7-22-41	13:10	5200	5300	75.2	75.5	77.2	47	.056	.291	.017	7.0	20	7.1	62	4.4	.....	15.9	0.48	2.9
2B	4311	7-22-41	13:10	5200	5300	75.2	75.5	77.3	40	.054	.283	.018	7.1	18	7.1	67	4.4	.....	16.4	0.47	3.3
1B	4311	7-23-41	13:09	5200	5300	75.2	75.5	77.2	20	.048	.....	.....	10.5	1	10.5	63	4.9	.....	16.4	0.47	3.4
3A	4435	7-21-41	13:11	5500	6000	74.5	75.1	77.9	30	.034	.....	.....	8.6	13	8.6	63	4.7	.....	16.6	0.62	4.6
3B	4435	7-21-41	13:11	5500	6000	74.5	75.1	77.8	15	.049	.....	.....	8.5	2	8.5	61	4.8	.....	16.4	0.62	5.4
4A	4435	7-18-41	13:13	5500	6000	74.5	75.6	78.4	28	.035	.306	.018	7.0	4	7.1	59	5.0	.....	15.5	0.56	5.7
4B	4435	7-18-41	13:13	5500	6000	74.5	75.6	78.6	37	.033	.330	.019	6.9	10	6.9	64	4.7	.....	15.6	0.61	5.7
5A	4307	7-13-42	13:15	4300	4200	74.3	75.6	77.3	25	.064	.228	.023	9.0	12	9.0	53	5.6	3.67	14.9	0.70	7.4
6A	4307	7-13-42	13:15	4300	4200	74.3	75.6	77.3	17	.072	.204	.020	9.3	13	9.3	50	5.5	3.70	14.5	0.68	7.6
7A	4307	7- 9-42	13:17	4400	3900	74.3	74.5	76.1	19	.062	.204	.023	10.0	8	10.0	55	5.4	3.58	15.3	0.81	8.0
6B	4307	7-13-42	13:15	4300	4200	74.3	75.6	77.3	30	.039	.243	.024	10.0	16	10.0	50	5.4	3.59	15.0	0.77	8.5
8A	4447	7-21-42	13:11	4100	3900	74.5	74.4	75.9	8	.082	.275	.019	11.8	12	11.8	57	5.2	3.57	15.9	0.93	8.5
8B	4447	7-21-42	13:11	4100	3900	74.5	74.4	75.9	6	.086	.283	.020	12.2	10	12.2	58	5.4	3.58	15.9	0.89	8.6
7B	4307	7- 9-42	13:17	4400	3900	74.3	74.5	76.2	20	.064	.173	.027	10.7	15	10.7	59	5.1	3.61	14.9	0.79	8.9
7C	4307	7- 9-42	13:17	4400	3900	74.3	74.5	76.1	26	.062	.251	.028	11.5	16	11.5	57	5.1	3.56	15.1	0.86	9.7
9A	4420	10-21-42	11:35	6800	6400	72.6	72.1	75.5	16	.045	.353	.024	6.3	9	6.3	66	4.7	3.40	14.6	0.98	11.3
10A	4420	10-21-42	11:35	6800	6400	72.6	72.1	74.5	12	.....	.....	.....	.....	12	5.9	51	5.0	3.40	14.2	0.98	12.1
9B	4420	10-21-42	11:35	6800	6400	72.6	72.1	75.2	19	.036	.204	.026	5.6	10	5.6	60	3.8	3.30	14.0	0.91	12.4
11A	4420	8-26-42	12:38	6900	7200	73.1	74.0	77.4	31	.027	.322	.029	5.5	4	5.5	63	3.3	3.33	15.0	1.12	12.6
10B	4420	8-26-42	12:38	6900	7200	73.1	74.0	77.6	....	.....	.....	.....	.....	7	5.6	66	3.4	3.31	14.9	1.12	12.7
11B	4420	8-26-42	12:38	6900	7200	73.1	74.0	77.5	18	.047	.330	.027	5.7	2	5.7	64	3.6	3.33	14.6	1.16	12.8

[1]Average two-hourly records six to two weeks prior to harvest of fruit [see Nightingale (1942)].

TABLE 2

Relatively High Ascorbic Acid Content Associated With Shade Treatment[1] and Lower Fruit Temperature

Plots	Field	Harvest date	Day length	Light rate		Air temp.		Fruit temp.	At floral differentiation					At time of harvest		Fruit					
				Weeks[2]		Weeks[2]		Weeks[2]	Leaf values												
				6	2	6	2	2	No. 1 color	Nitrate N	K	P	Plant wt.	No. 1 color	Plant wt.	Exposure	Weight	pH	Brix	Acid as citric	Ascorbic acid
			hr.	*f.c.*	*f.c.*	°*F.*	°*F.*	°*F.*	*pct.*	*pct.*	*pct.*	*pct.*	*lb.*	*pct.*	*lb.*	*pct.*	*lb.*			*pct.*	
Check	4307	7- 9-42	13:17	4400	3900	74.3	74.5	77.3	20	.064	.173	.027	10.7	15	10.7	59	5.1	3.61	14.9	0.79	8.9
Shade	4307	7-13-42	13:15	4300	4200	74.3	75.6	76.4	25	.064	.228	.023	9.0	1	9.0	24	5.6	3.41	14.9	1.12	11.0

[1]From May 29 until the fruits were harvested on July 15, a lath shelter that shut out 50 per cent of the sunlight was placed over the plants of the shade plot. [2]Average two-hourly records six and two weeks prior to harvest of fruit.

sets of plants were well supplied with phosphorus and potassium as shown by chemical analyses of index leaves. Plants in B produced three tons more fruit per acre than A and had greater reserves of nitrate. The plants were conspicuously different in quality, also in size. Thus, from a practical standpoint the differences in nitrogen fertilization were extreme. It will be noted that this experiment is very similar to Experiment I, both in design and in results.

Experiment IV: Conducted in Field 4435, this experiment was essentially of the same design as Experiment II. The general condition of the plants of the A and B groups was the same in both experiments. No effects of micro-element fertilization on plants of B were noted.

Experiment V: Conducted in Field 4307, this experiment was designed to determine effects of a 50-per cent reduction in sunlight 45 days before harvesting the fruit. At the time of floral differentiation all the plants were high in carbohydrates. Plants of this type were purposely selected for the experiment in order that the plants to be shaded would not become seriously deficient in carbohydrates. All plants received 440 lb. of nitrogen per acre. Plots A were maintained in full sunlight, and the plants of Plots B were shaded by means of a lath structure to give a 50-per cent reduction in light. The fruit of both shaded and check plots was of excellent quality and highly translucent (i.e., the flesh cells had become thin-walled and the intercellular spaces were filled with juice). The shading did not noticeably influence fruit size although a definite increase in acidity and ascorbic acid content was noted (see later discussion and Table 2).

Experiment VI: Also conducted at Field 4307, this experiment was designed to study effects of an excess supply of nitrogen. Plants of Plots A received 400 lb. of nitrogen per acre, and those of B, 820 lb. No phosphorus or potassium fertilization was used although leaf analysis indicated the presence of fairly adequate amounts of these minerals. There were rather extreme differences between A and B in plant quality at the time of floral differentiation, plants of A being much lower in nitrates and higher in carbohydrates than those of B. Nevertheless, at the time of fruit harvest there was no measurable difference in plant quality or in fruit weight in the case of those plants for which records were made. This fact, that the extreme differences between the plots at the time of differentiation did not prevail at harvest, is not entirely clear. It may be that the lack of differences in yield was associated with a limited fruitfulness in A because of too little nitrogen and limited fruitfulness in B because of too much nitrogen, Nightingale (1942). The lack of differences in fruit quality may have resulted from the fact that the fruit selected in B was ripe at the time A was ready to harvest. Fruit in B ripened more slowly than in A, and in order to harvest both plots on the same date considerable selection was necessary in B to obtain ripe fruit.

Experiment VII: This experiment was conducted in Field 4307 and was divided into three parts. Plots A received 440 lb. of nitrogen per acre and no phosphorus or potassium; B received 460 lb. of nitrogen per acre and no phosphorus or potassium. Plots C were supplied with 440 lb. of nitrogen per acre and 400 lb. of potassium. Plants of A were in an experimental area about 100 yards from that of B and C and received a more

liberal water supply. Apparently because of previous fertilization practices, Plots A were deficient in phosphorus and in potassium, while Plots B and C were not deficient in phosphorus. Thus, the plants of the three treatments represented three states of deficiency. A was deficient in phosphorus and potassium; B was deficient in potassium; and C had liberal amounts of both phosphorus and potassium. All three lots received liberal supplies of nitrogen. Plot B received 20 lb. more of nitrogen per acre than C in the hope that by this slight increase the nitrogen uptake of the plants in the two plots would be equalized. The reason for this is that the presence of potassium fertilizer in Plots C, as usual in the case of pineapple, increased the ability of the plants in those plots to absorb nitrate. Differences in plant quality between A plots and the B and C plots were apparent at the time of floral differentiation and at harvest, plants of A showing definite symptoms of phosphorus deficiency. Differences between B and C were not great, although the latter contained more potassium.

Experiment VIII: Conducted in Field 4447, all plants of this experiment were liberally fertilized with the necessary macro-elements. A received 10 sprays with a four-per cent solution of iron sulfate; B received 32 sprays with four per cent of iron sulfate. In A the sprays were mostly applied early in the life of the plant. Plants of Plot B produced nearly a ton per acre more fruit than A. The latter plants, as judged by appearance, were much more extreme in degree of iron deficiency than would ever occur in good commercial fields.

Experiment IX: This was conducted in Field 4420. All plants received 300 lb. of nitrogen per acre. A received 450 lb. of K_2O and 200 lb. P_2O_5. B received neither potassium nor phosphorus fertilization. The most conspicuous difference in plant composition was in the much higher range of potassium values of A as compared with B. With the increased potassium there was also an increase in nitrate in Plots A. However, the latter fact was not associated with apparent deficiencies in carbohydrate reserves. Plants of A were larger and had greater yield of fruit than B. The quality of the fruit in the two cases was, as nearly as could be determined, the same. While phosphorus fertilization differed, no differences in content of phosphorus in the plants were found.

Experiment X: Conducted in Field 4420, this experiment was designed to determine the possible effects of season on vitamin content. All plants were liberally supplied with nitrogen, phosphorus, and potassium. Harvest dates are indicated in the table.

Experiment XI: This experiment was conducted in Field 4420. Plots A received 120 lb. and B received 320 lb. of nitrogen per acre. In addition to the above differences in nitrogen fertilization, plants of B were supplied with 150 lb. more K_2O than A. Plants of A were clearly deficient in nitrogen as compared with B when judged by color, nitrate content, and size of plant and fruit. Fruit quality was about the same in both plots. This field was noticeably cooler at the time the fruits were harvested.

RESULTS

Data on experimental results (Tables 1 and 2) have been arranged in ascending order of ascorbic acid values regardless of the source of the

material or the experiment from which they came. It may be noted that the ascorbic acid content varied from 2.9 to 12.8 mg. of ascorbic acid per 100 c.c. of juice. Arranging the data in this manner, it is possible by inspection of the table to ascertain that most of the other data recorded here are not correlated with ascorbic acid values. However, it seems obvious that there is a fairly close correlation between air temperature, total acid (as per cent citric), and ascorbic acid content. The total-acid values range from 0.47 to 1.16 per cent, and the various values increase almost directly with the increase in ascorbic acid. The correlation coefficient between total acidity and ascorbic acid content was found to be 0.956, which is highly significant. In general, the higher ascorbic acid values were obtained in those fields with the lowest air temperature during six weeks previous to harvest, and air temperature was inversely correlated with ascorbic acid content with a correlation coefficient of —0.767. Although pH values were not determined on a number of the samples, there seemed to be a decrease in pH with increasing ascorbic acid and total acid values, as would be expected. There was a tendency for Brix values to decrease with increasing ascorbic acid, but the decrease was not great nor was it consistent. It is apparent that the fields from which the samples were taken were correlated with ascorbic acid values since there is no overlapping of the samples from the different fields, with the single exception that samples from Field 4447 fall in the group of those samples from 4307; and it may be noted these two fields were subjected to very similar climatic conditions just prior to harvest of fruit. There is little, if any, indication that experimental treatments produced very great effects upon ascorbic acid content, with the single exception of Experiment V. In this experiment, the shaded plants had appreciably higher ascorbic acid values than the unshaded plants. Shading the plants resulted in an increase of 3.6 mg. per 100 c.c. It is obvious that the magnitude of the influence of fertilizer treatment is not great as compared with the influence of climatic factors. It seems apparent that there is no correlation in these data between ascorbic acid content and the following values: harvest date, day length, fruit temperature, leaf color, leaf nitrate, leaf potassium, leaf phosphorus, plant weight, exposure to sun, and fruit weight.

DISCUSSION

The most significant result obtained in these experiments was the close correlation between ascorbic acid content and total acidity of the juice. This has been found to be consistently true for both fresh and processed juice over a period of several years. The fact that one field produces more acid fruit than another is especially interesting, and one would naturally expect that these differences in acidity would be correlated with differences between the soils or the climatic environmental factors prevailing at the two fields. While none of the data presented here indicate what the differences between the fields might be, which would result in differences in acidity and in ascorbic acid content, it is well known that during the relatively cool winter months fruit is comparatively high in acidity and ascorbic acid.

While the evidence here indicates that nitrogen fertilization may result in a slight increase in ascorbic acid content, the magnitude of these effects is slight. Variations in fertilizer practices much greater than any which

would be expected to occur in a commercial field seem to be of little significance in determining the ascorbic acid content of pineapple juice. Such results are consistent with many of those obtained with other plants, Maynard and Beeson (1944). Differences in ascorbic acid content of fruit from one field as compared with another were fairly great. It is difficult, with the present data, to relate these differences with any known variation in soils. However, previous results obtained with tomatoes by Hamner, Lyon, and Hamner (1942) indicated that variations in the location at which the plants are grown produced differences in ascorbic acid content and that these differences could not be attributed to soil variations. The fact that the fertilizer treatments employed here had little effect on ascorbic acid values might serve as a further indication that the field differences were not the results of soil factors. Close examination of what data is available with respect to climatic factors, however, indicates that air temperature may be one of the factors responsible for these variations.

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ORGANOLEPTIC SIGNIFICANCE OF KIDNEYS IN POULTRY

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When dressed poultry is subjected to high temperatures for a short time or held at favorable cooler temperatures for a prolonged period, deteriorative changes occur which lower quality. At least part of this deterioration in dressed poultry has its source in the visceral organs, particularly the intestines. It has not been demonstrated clearly whether the kidneys do or do not contribute to the development of off-odors and off-flavors. Nickerson and Fitzgerald (1939) pointed out that workers in the trade sometimes describe the odor of certain drawn birds as a "kidney odor." Stewart, Lowe, and Morr (1941), in observing post-mortem changes in New York dressed poultry held at 1.7°C.(35°F.), reported that after 48 hours the kidney area had a strong characteristic off-odor which seemed to spread gradually to the hip and wing joints of the carcass. It was not indicated whether this off-odor was due to the kidneys or the intestines.

Because definite data were not available, experiments were conducted to determine if the kidneys are a factor in lowering the quality of poultry.

EXPERIMENTAL PROCEDURE

In this study three separate experiments were conducted. The details of each varied and consequently the procedure followed for each experiment will be described separately.

Twenty fryer-size, battery-raised birds, dressed in the conventional manner and chilled overnight in air at 3.3°C.(38°F.), were used in Experiment 1. The birds were then eviscerated in the usual manner except that the kidneys were not removed from one half of them. Next, the birds were wrapped in cellophane and frozen at —28.9°C.(—20°F.) and held in the freezer for one week. Immediately upon removal from freezer storage, two birds with kidneys and two without were cooked and taste-scored for control purposes. The remainder of the lot was placed in a household refrigerator. Subsequently, two samples similar to the controls were cooked and tested for aroma and flavor after one, six, nine, and 13 days of storage, respectively.

In Experiment 2, 30 farm-raised, heavy, fryer-size birds were used. The dressed poultry was rapidly chilled and frozen in a sharp freezer. They were held in the freezer for two weeks, then placed in water, and, upon defrosting, were eviscerated immediately and wrapped in cellophane. Three birds with kidneys and three without were then again frozen for use as controls later. The other 24 birds were held at 3.3°C. for two days. Immediately upon removal from the 3.3°C. cooler, three birds with kidneys and three without were frozen. The remaining birds were transferred to a 10°C.(50°F.) cooler. Subsequently, after three different storage periods (two, four, and seven days, respectively) three birds with kidneys and

three without were frozen. The eviscerated birds were all frozen in still air at —28.9°C. Within the next month on three different arbitrarily chosen days one bird with kidneys and one without, representing each of the five lots, were defrosted, cooked, and scored.

Experiment 3 was designed to determine whether long periods of freezer storage of fryer-size chickens and fowl would result in the development of off-flavors or odors because of the presence of the kidneys.

Forty chickens were used in this experiment. Ten birds, five with kidneys, and five without, were prepared in four different ways as follows:

(1) Eviscerated immediately after dressing (warm evisceration).
(2) Eviscerated after air-chilling at 3.3°C. for 24 hours.
(3) Eviscerated after air-chilling overnight at 3.3°C. and storage for three days at 10°C.
(4) Eviscerated after chilling overnight at 3.3°C., freezing, and defrosting.

Immediately after evisceration the fryers were frozen at —28.9°C. and, except for the controls, were then held at —23.3°C.(—10°F.). Promptly after freezing, two control birds, one with kidneys and one without, from each of the four groups were defrosted, cooked, and tested. Subsequently, after storage periods of one, four, six, and nine months, two more birds from each group were removed, defrosted, cooked, and scored.

In addition to the above studies pertaining to chickens, observations were made also on fowl in Experiment 3. Two with kidneys and two without kidneys were air-chilled overnight, frozen, and held in freezer storage for zero, one, four, six, and nine months. The birds were stewed and then scored for general aroma and flavor of the dark and white meat.

All chickens were roasted and were considered cooked when the interior thigh tissues reached a temperature of 88°C.(190°F.). Each bird was scored by two to four individuals, all with previous experience in evaluating poultry quality. In the scoring the birds were given numerical ratings. An arithmetic average of the individual evaluations for each bird was made. The aroma was evaluated immediately after removal from the oven as being (5) very desirable; (4) desirable; (3) slightly desirable; (2) slightly undesirable; and (1) undesirable. The flavor of the white and dark meat then was scored separately as being (5) excellent; (4) very good; (3) good; (2) fair; and (1) poor.

DISCUSSION OF RESULTS

The results secured in connection with Experiment 1 (Table 1) demonstrated that the presence of kidneys made no essential difference in the aroma or flavor of poultry held for varying lengths of time in the household refrigerator. Quality was lowered in both lots owing to the extended holding period at relatively high temperatures.

In Experiment 2 (Table 2) where the poultry was held for two days at 3.3°C. and then for additional but varying lengths of time at 10°C., the results also demonstrate that there is no essential difference in the quality of cooked birds with or without kidneys. Here again there was a very marked impairment of quality for both groups of birds as a result of the extended holding period and the relatively high temperature.

TABLE 1

Summary of Results of Experiment 1 Comparing Quality of Eviscerated Poultry, With and Without Kidneys, Held in a Household Refrigerator for Varying Lengths of Time

Days held in refrigerator	Average organoleptic scores for two birds					
	General aroma		Flavor of dark meat		Flavor of white meat	
	Kidney in	Kidney out	Kidney in	Kidney out	Kidney in	Kidney out
0	4.25	4.00	4.25	3.88	4.50	4.25
1	4.66	4.33	4.50	4.33	4.66	4.33
6	4.66	4.56	4.08	4.33	4.33	4.33
9	3.50	4.33	2.00	3.16	1.83	3.16
13	2.75	2.75	1.38	1.50	1.50	1.63

TABLE 2

Summary of Results of Experiment 2 Showing Organoleptic Scores for Cooler-Held, Eviscerated Poultry With and Without Kidneys

Days held in cooler		Total days held in cooler	Average organoleptic scores for three birds					
			General aroma		Flavor of dark meat		Flavor of white meat	
38°F.	50°F.		Kidney in	Kidney out	Kidney in	Kidney out	Kidney in	Kidney out
0	0	0	3.33	4.50	4.00	4.15	4.65	5.00
2	0	2	3.50	3.37	3.25	3.62	3.15	3.00
2	2	4	2.25	2.87	2.75	2.62	2.65	3.45
2	4	6	3.62	2.62	3.00	2.25	2.75	2.90
2	7	9	1.25	1.37	1.37	1.37	2.00	1.95

TABLE 3

Summary of Results of Experiment 3 Showing Organoleptic Scores for Eviscerated Poultry, With and Without Kidneys, Subjected to Varying Cooler and Freezer Storage Conditions

Factors tested	Months in freezer storage, —10°F.	Methods of handling							
		Warm eviscerated		Overnight chilled at 38°F., eviscerated		Frozen, defrosted, eviscerated		Held 3 days at 50°F., eviscerated	
		Kidney in	Kidney out	Kidney in	Kidney out	Kidney in	Kidney out	Kidney in	Kidney out
Aroma	0	4.00	4.33	3.66	3.66	4.00	4.33	1.33	2.00
	1	4.66	4.33	4.33	4.66	4.33	4.33	1.33	1.33
	4	4.00	4.00	3.75	4.00	3.50	3.75	1.50	2.00
	6	3.62	3.75	4.50	4.00	4.50	3.75	2.50	1.87
	9	3.50	3.50	4.50	4.00	3.00	3.50	1.00	1.00
Flavor of dark meat	0	4.33	4.33	4.33	4.33	4.33	4.33	3.33	3.66
	1	4.33	4.33	4.00	4.00	4.00	4.33	2.33	2.33
	4	3.75	4.00	3.50	3.50	3.25	3.00	2.00	2.00
	6	3.12	3.12	4.37	4.12	4.00	3.87	2.12	2.25
	9	4.00	4.00	4.50	4.50	3.00	3.50	1.50	1.00
Flavor of white meat	0	4.33	4.66	4.00	4.00	4.00	4.00	3.33	3.66
	1	4.33	4.66	4.00	4.00	4.00	4.33	2.33	3.00
	4	3.75	3.75	3.75	3.75	3.75	3.75	2.25	2.25
	6	3.87	3.62	4.37	4.37	3.87	3.87	3.50	2.87
	9	4.00	4.00	4.00	4.00	4.00	4.00	2.50	2.50

Results of Experiment 3 (Table 3) demonstrate that kidneys do not impair quality when chickens are subjected to freezer storage at —23.3°C. The chickens eviscerated as warm, chilled, or defrosted poultry were distinctly superior to poultry held for three days at 10°C. prior to evisceration. There was no indication of an organoleptic difference between the two lots of fowl.

In Experiments 1 and 2, the kidneys for each fryer-size chicken were inspected separately for aroma and flavor. In each instance, the kidneys remained palatable and had a flavor very much like chicken liver. The kidneys of the fowl were also tasted and scored, the results being similar to those recorded for chickens. The quality of the kidneys, even when subjected to unfavorable holding conditions, did not seem to be impaired any more than that of the other edible tissues.

In Experiments 1 and 2, bacteriological counts per gram of tissue for the dark meat and for the skin were made for birds with kidneys and without kidneys. The total counts indicated that there was no difference in the number of bacteria contained in the skin or dark meat for the two groups. In making these bacteriological comparisons Difco nutrient agar plates were incubated at 20°C.(68°F.) for three days.

The results of the three experiments indicate that kidneys have no effect on the quality of poultry subjected to various holding conditions, including prolonged freezer storage.

This study is believed to demonstrate that the retention of kidneys in otherwise eviscerated birds is not likely to impair the quality of the product. It appears that most of the objectionable odors and flavors that may develop in poultry are due to presence of the intestines in the body cavity and not to presence of the kidneys.

SUMMARY

Under the procedures followed in the present experiments the kidneys did not adversely affect the quality of otherwise eviscerated poultry.

There was a gradual deterioration or lowering of quality in dressed birds held at temperatures considerably above freezing which, in general, was proportional to the length of time that the product was subjected to the adverse conditions.

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